Universitat de Girona

Facultad de Medicina

Sensitivity and specificity of fine needle aspiration guided by ultrasounds to detect the sentinel lymph node. Its impact in axillary treatment of breast cancer.

Cross sectional study

FINAL GRADE PROYECT

JANUARY 2017

BREAST PATHOLOGY UNIT

AUTHOR: Elisabet Barahona San Millán

TUTOR: Dra. Ester Vila Camps

I would like to thank Dr. Josep Trueta's Hospital Breast Pathology Unit for their continued support and encouragement: Dra. Ester Vila, my final grade project tutor; Dra. Alexandra Bonmatí and Dr. Francesc Tuca. I would also like to thank all the people who got involved with this project, without their generous support it would not have been possible.

Finally this project is dedicated to dear my family, my greatest support.

INDEX

1. ABREVIATIONS....4
2. ABSTRACT....6
3. INTRODUCTION....7
   3.1 Background....7
   3.2 Justification....18
4. BIBLIOGRAPHY....20
5. QUESTION....24
6. HYPOTHESIS....24
7. OBJECTIVES....24
8. MATHERIAL AND METHODS....25
   8.1 Study design....25
   8.2 Participants....25
   8.3 Inclusion criteria....25
   8.4 Exclusion criteria....25
   8.5 Sample selection....26
   8.6 Sample size....26
   8.7 Principal Variable....26
   8.8 Covariables....27
9. PROCEDURES....27
10. METHODS OF DATA COLLECTION....31
11. STATISTICAL ANALYSIS....34
12. ETHICAL ASPECTS....35
13. LIMITATIONS OF THE STUDY....36
14. WORK PLAN....37
    14.1 Time Schedule....39
15. FEASIBILITY....40
16. BUDGET....41
17. CLINICAL AND HEALTHCARE IMPACT....42
18. ANNEXES....44
    18.1 Annex I....44

18.2 Annex II……………………………………………………………………………………………………..49

18.3 Annex III………………………………………………………………………………………………………51

18.4 Annex IV……………………………………………………………………………………………….……..54

18.5 Annex V……………………………………………………………………………………………………...56

18.6 Annex VI…………………………………………………………………………………………….……….57

18.7 Annex VII……………………………………………………………………………………………….…….60

1. ABREVIATIONS

-ALND: axillary lymph node dissection

-APBI: accelerated partial breast irradiation

-AWS: axillary web syndrome

-BCS: breast conservative surgery

-BCT: breast-conserving therapy

-BMI: body mass index

-CM: coordination meeting

-DCIS: ductal carcinoma in situ

-ER: estrogen receptors

-FNA: fine needle aspiration

-H&E: hematoxylin and eosin

-HR: hormone receptor

-HT: hormone therapy

-IH: immunohistochemistry

-LCIS: lobular carcinoma in situ

-LNs: lymph nodes

-MRI: magnetic resonance imaging

-NHBF: non-hiliar blood flow

-NPV: negative predict value

-OSNA: one-step nucleic acid amplification

-PPV: positive predict value

-PR: progesterone receptors

-ROM: range of motion

-RT: radiotherapy

-SLN: sentinel lymph node

-SLNB: sentinel lymph node biopsy

-US: ultrasounds

-US-FNA: fine needle aspiration guided by ultrasounds

-WBRT: whole breast radiation therapy

2. ABSTRACT

Background Axillary lymph node affection in breast cancer is an important prognosis factor. The treatment of the axilla by Axillary Lymph Node Dissection (ALND) depends on the staging which is established by some complementary techniques. The presurgery staging of axillary affection is done by FNA-US and if it's negative the SLNB is done. ALND is an aggressive procedure that nowadays is being restricted more and more. Actually in the Z0011's era some patients with a positive SLNB aren't undergoing the ALND. However patients with positive FNA-US must suffer the ALND procedure and its complications. If it's proved that the positive lymph nodes detected by ultrasound are the positive sentinel lymph nodes. Early breast cancer patients with one or two positive lymph nodes by FNA-US would be able to spare the ALND as it's done now with positive SLNB according to Z0011 trial.

Purpose The goal of the present work is to demonstrate the agreement of two diagnosis test: Sentinel Lymph Node Biopsy (SLNB) and Fine Needle Aspiration guided by Ultrasounds (US-FNA). Our principal variable is axillary metastasis. We will do an estimation of the proportion of positive lymph nodes by US-FNA that are the positive sentinel lymph nodes. So that patients would undergo same treatment decisions about sparing ALND.

Design A diagnosis test study with a cross-sectional design carried out in a tertiary referral hospital in Girona within a Breast Pathology Unit in the period of one year.

Participants Women diagnosed of early and non-aggressive breast cancer that undergo US-FNA for the locoregional lymph nodes staging.

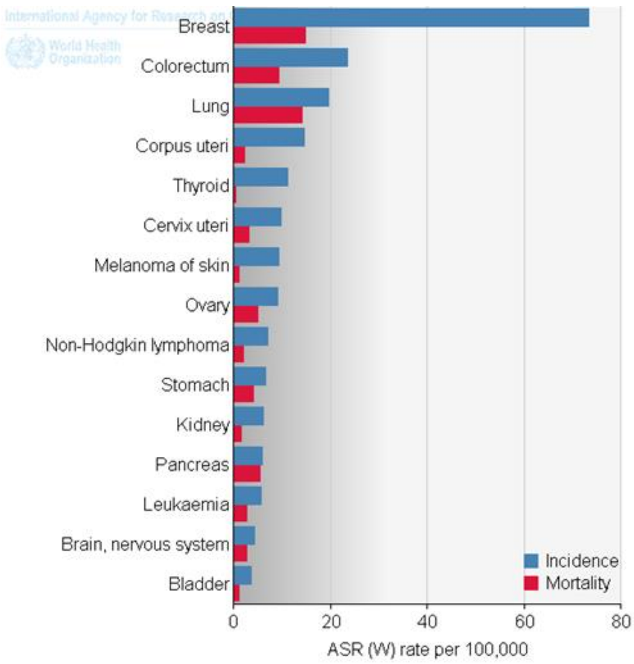
Key Words Early stage of breast cancer; Ultrasound guided-Fine Needle Aspiration(US-FNA); Sentinel Lymph Node Biopsy(SLNB); Axillary clearance;

3. INTRODUCTION

3.1 BACKGROUND

Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in 2012(graphic 1) (this represents 25% of all cancers).Breast cancer is the fifth cause of death from cancer overall (522,000 deaths). While it is the most frequent cause of cancer death in women in less developed regions, it is the second in more developed regions after lung cancer.(1)

Graphic 1: Estimated age-standardized incidence and mortality rates: women.



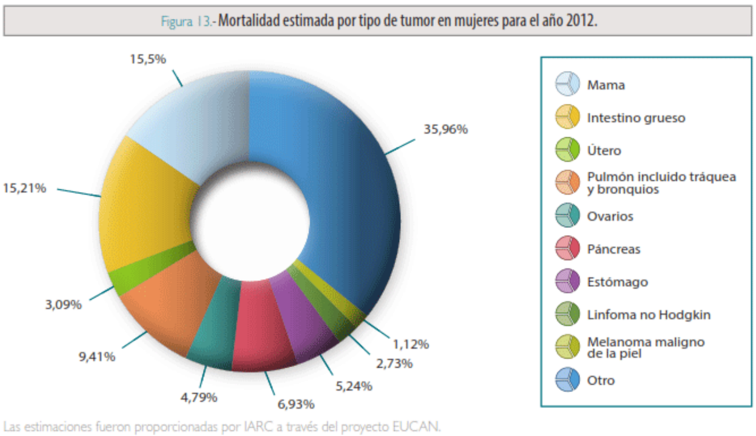
Resource:*Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F.GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on day/month/year*.(1)

In Europe breast cancer represents the third cause of cancer death after colorectal cancer and lung cancer. Among Spanish women breast cancer is the first cause of cancer death. In Spain is diagnosed about 26,000 of breast cancer cases per year. It represents 29% of all cancers in our country. Either the new cases or the incidence is increasing slowly in Spain and in the world. It is probably because of the ageing of the population and thanks to an earlier diagnosis.(2)

The risk estimated to have breast cancer along the life is about 1 per every 8 women.

Breast cancer was the leading cause of death in women in 2012 (6,075 cases), followed by colon cancer (5,985 deaths), lung (3,688 deaths), pancreas (2,717 deaths) and stomach (2,054 deaths).(3)

Graphic 2: Estimated mortality by tumor type in women for 2012



Resource: Sociedad Española de Oncología Médica(SEOM)(3)

Early detection of cancer thanks to mammography screening, and the improved treatments, made the incidence of breast cancer increased, although mortality is decreasing. Any way breast cancer is still the leading cause of cancer related deaths in European women. (4)

The globally survival rates of breast cancer after 5 years is 89%. But it depends on the estadio which it is diagnosed; The overall survival in estadio I is 98% while in estadio III it decrease to 24%.(4)

The main risk factors include: genetic predisposition, exposure to estrogens, ionizing radiation, low parity and history of atypical hyperplasia. The most important risk factor is the age, so that only a quarter of breast cancer occurs before age 50.(5)

In the province of Girona from 2007 to 2009 there are 109,3 cases of breast cancer per 100.000 women per year. The brute rate of breast cancer mortality in women has been 28,2 per 100.000 women year. In 2013 and 2014 there were 389 cases of breast cancer each year.(6)

The incidence is increasing due to the introduction of breast cancer screening and the ageing of the population. According to European guidelines the mammography screening consists on regular mammography every two years in women aged 50-69 years. Women with familiar breast cancer history with or without proven mutation need a different screening. Magnetic resonance imaging (MRI) every year alternating with mammography every six months. This high risk group starts the screening 10 years younger than the youngest case in the family. It can be use the ultrasound (US) too, although there isn't consensus. (5)(7)

There are risk factors related with breast cancer. The most important are: age, genetic predisposition (people who is positive for BRCA1 , BRCA2); exposure to estrogens (endogenous and exogenous);ionizing radiation; low parity ;a history of atypical hyperplasia .There are other factors that can contribute to the increasing risk like: lifestyle, obesity, alcohol.(8)

Breast cancer in males represents just 1% of cases. The major risk factors are disorders carrying hormonal imbalances (especially gynecomastia and cirrhosis), radiation exposure and a positive family history and genetic predisposition.(5)

Thanks to screening the detection of breast cancers at pre-clinical stage has highly increased. That makes an important prognosis factor, that increase as well the survival rate after the suitable treatment

There is a classification depending on the different types of breast cancer (Annex I). We can mainly differentiate between the in situ tumors and the infiltrative ones.

The term "carcinoma in situ" means abnormal epithelial cells that have not invaded nearby tissues. On the other hand the invasive tumor cells exceed the basement membrane of the ducts into the surrounding breast tissue. Inside the in situ tumor, there are two types: the lobular and the ductal one. The most common is the ductal one.(5)

Another classification is defined by immunohistochemical (IHC) criteria. In clinical practice it's done an approaching to molecular subtypes from IHC of hormone receptors, Her 2 and Ki-67.According to this there are four subtypes:(5)(9)

-Luminal A-like: positive ER, positive PR >20%, negative Her-2 and Ki-67 <20%

-Luminal B-like: positive ER, PR<20%, positive or negative Her-2 and Ki-67>20%

-Her-2 overexpression: positive Her-2, negative ER and PR

-Triple-negative: negative ER, negative PR and negative Her-2

DIAGNOSIS

The diagnosis of breast cancer is based on clinical examination in combination with imaging and confirmed by pathological assessment:(5)

- Clinical examination consist on bimanual palpation of breast and loco regional lymph nodes and assessment for distant metastases (bones, liver and lungs)
- Imaging includes: bilateral mammography, ultrasounds of regional lymph nodes and MRI in special cases (ultrasound lymph nodes inconclusive, familiar breast cancer associated with BRCA, breast implants, lobular cancers, multifocality, clinical-radiological discordance…). In order to classify a tumor like suspicious of malignancy in radiology it is used the BIRADS classification (Annex III)(10)
- Pathological assessment of the primary tumor with a core needle biopsy(to classify the histological type, the grade and the hormone receptors) and a fine needle aspiration guided by ultrasound(US-FNA) to obtain cytology/histology of axillary nodes, if involvement is suspected(by clinical examination or ultrasounds). Patients with clinically and imaging negative axilla, it is indicated to carry out sentinel lymph node biopsy (SLNB).

The final pathological diagnosis should be made according to TNM classification(Annex I) and the pathological report (histological type, grade(Annex II), immunohistochemical(IHC) evaluation of :estrogen(ER) receptors, progesterone(PR) receptors and HER2 receptors)(5)

-The most important prognostic factor in early breast cancer are:(8)

- expression of ER/PR, HER2
- proliferation markers(Ki-67, p53, bcl-2, cadherina-E),
- number of involved regional lymph nodes(N),
- tumor histology,
- the tumor size (T),
- grade and presence of peritumoral vascular invasion
- in patients undergoing breast-conserving therapy(BCT) , the recurrence risk is related to the status of the surgical margins.

-Extension study consists on chest and abdominal CT scan and a bone scintigraphy. It is performed in patients with: clinically positive axillary nodes, large tumors (>5cm), aggressive biology or clinical signs, symptoms or laboratory values suggesting the presence of metastases.(5)

Local treatment

There are two possibilities of breast surgery treatment: breast-conservation surgery (BCS) and the mastectomy. (11)

<u>-Breast conservation surgery</u> (BCS) started being performed about 30 years ago. It consists on a wide local excision and radiation therapy (RT). Oncoplastic procedures can result in better cosmetic outcomes. Especially in patients with:

- Large breasts,
- A less favorable tumor/ breast size ratio
- A cosmetically challenging location of the tumor within the breast (central or inferior).

A careful histological assessment of resection margins is essential (>2mm). Marking the tumor bed with clips is a standardized way to facilitates accurate planning of the radiation boost field.

<u>-Mastectomy</u> is still carried out in patients with: large tumor size relative to breast size; multicentric tumor; inability to achieve negative surgical margins after multiple resections; prior radiation to the chest or other contraindication to RT; patient’s choice. Breast reconstruction should be available for these patients.(12)

Radiation Therapy(5):

<u>Whole breast radiation</u> (WBRT) reduces the 10 year risk of any first recurrence (locoregional and distant) by 15%. That’s why RT after BCS is strongly recommended. It is indicated for patients who have unfavorable risk factors for local control such as: age<50; grade 3 tumors; extensive DCIS; vascular invasion or non-radical tumor excision.

<u>Accelerated partial breast irradiation only</u> (APBI): most of the local recurrences occur nearby the primary tumor. That’s the reason why this technique focuses the irradiation to the vicinity of the primary tumor site. This is a treatment option for people older than 50´s

with: unicentric and unifocal tumor, node-negative margins, especially if they will receive adjuvant hormonal treatment.

Regional irradiation: Locoregional RT includes the chest wall and all regional lymph nodes. It is useful especially for patients with involved axillary lymph nodes. It is not described which part of the regional lymph nodes is most important to irradiate.

Axillary Staging:

Regional lymph node status remains one of the strongest predictors of long-term prognosis in primary breast cancer as well as a factor determining treatment.

The breast lymphatics drain mainly through three routes: axillary, transpectoral, and internal mammary. Internal mammary lymph nodes are coded as axillary lymph nodes for staging purposes. Supraclavicular lymph nodes are classified as regional lymph nodes for staging purposes. Metastasis to any other lymph node, including cervical or contralateral internal mammary lymph nodes, is classified as distant (M1).(13)(5)

The regional lymph nodes are:

- Axillary (ipsilateral): interpectoral nodes and lymph nodes along the axillary vein and its tributaries that are divided into the following levels:
    - -Level I (low-axilla): lymph nodes lateral to the lateral border of pectoralis minor muscle.
    - -Level II (mid-axilla); lymph nodes between the medial and lateral borders of the pectoralis minor muscle and the interpectoral lymph nodes.
    - -Level III (apical axilla): lymph nodes medial to the medial margin of the pectoralis minor muscle.
- Internal mammary (ipsilateral): lymph nodes in the intercostal spaces along the edge of the sternum in the endothoracic fascia.
- Supraclavicular: lymph nodes in the supraclavicular fossa, a triangle defined by the omohyoid muscle and tendon (lateral and superior border), the internal jugular vein (medial border), and the clavicle and subclavian vein (lower border). Adjacent lymph nodes outside of this triangle are considered to be lower cervical nodes (M1).

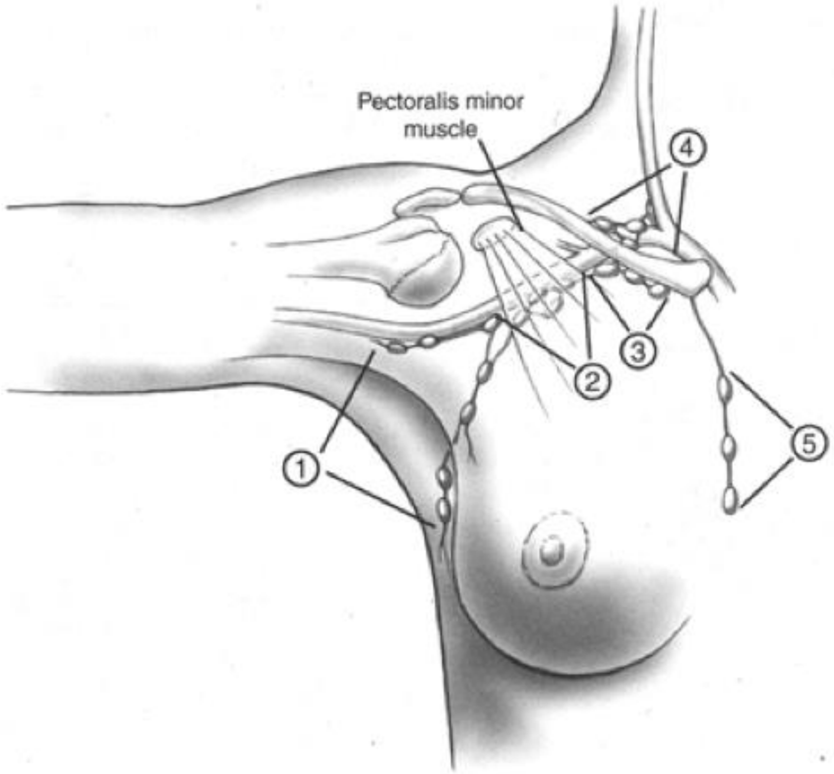


Figure 1*: Schematic diagram of the breast and regional lymph nodes. 1 Low axillary, Level I; 2 Mid-axillary, Level II; 3 High axillary, apical, Level III; 4 Supraclavicular; 5 Internal mammary nodes*.(13)

The axilla is frequently examined in imaging studies performed in patients with invasive breast cancer. Imaging plays an important, although limited, role in preoperative clinical staging of axillary disease. Axillary Ultrasound combined with Fine Needle Aspiration(US-FNA) can have a high level of accuracy in axilla evaluation. If false-positive findings from axillary imaging leads the surgeon to perform an unnecessary ALND. And if ambiguous axillary imaging findings result in too many negative percutaneous biopsies, then axillary imaging will become clinically irrelevant. This can be avoided by understanding the appearances of normal and metastatic nodes. Using all imaging modalities and through accurate preoperative sampling of suspicious lymph nodes we can reduce the amount of false positive and know which are the right nodes that we have to aspirate.(14)(15)(16)

US Findings	Comments
Diffuse cortical thickening	Cortical thickness> 3mm, relatively nonspecific, can be seen in reactive nodes
Focal cortical bulge	Should be distinct, otherwise less specific; more specific if associated with another finding such as NHBF(non-hiliar blood flow)
Lymph node size	>10 mm if associated with other suspicious signs
Eccentric cortical thickening	Should be distinctly eccentric, otherwise less specific
Rounded hypoechoic node	High specificity in the setting of invasive cancer
Complete or partial effacement of the fatty hilum	High specificity in the setting of invasive cancer
NHBF on color Doppler images	Nonspecific unless combined with another finding, such as effacement of the fatty hilum
Complete or partial replacement of the node with an ill-defined or irregular mass	High specificity
Microcalcifications in the node	Should correlate with microcalcifications in the primary tumor

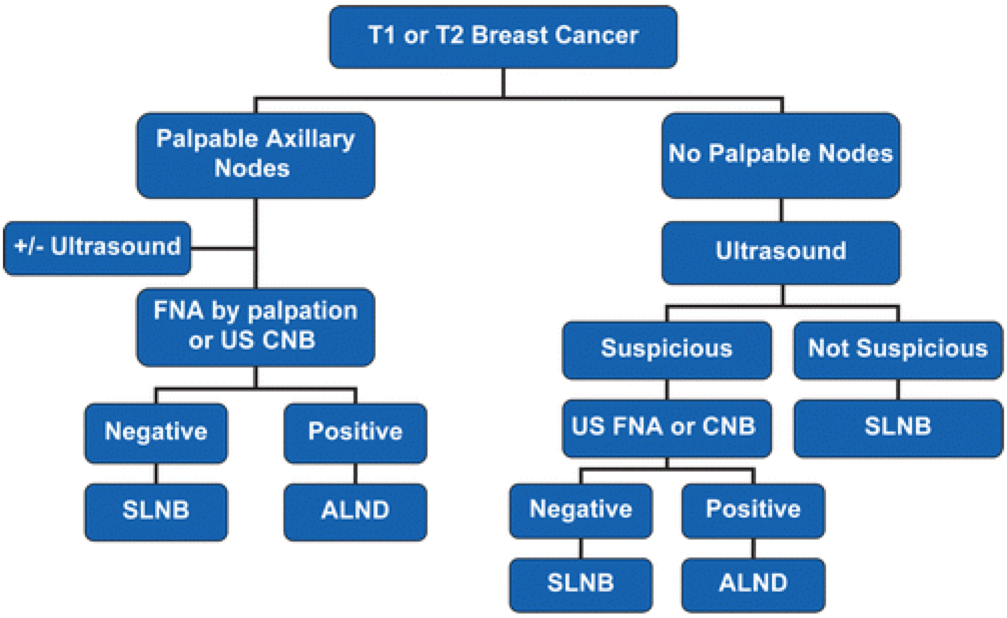
(15)(16)(17)

After doing the Fine Needle Aspiration the specimen is carried to Pathology. The histological diagnosis is made by processing the specimens with hematoxylin-eosin (H&E) and Immunohistochemical(HI) analysis when the result with H&E is negative.(18)

Diagnosis of axillary metastasis is also important for determining the need for systemic chemotherapy as well as radiation therapy. Axillary lymph node dissection (ALND) is the definitive method to diagnose axillary metastasis (gold standard). But it carries a lot of false positive patients over treated. Now sentinel lymph node biopsy (SLNB) has supplanted this procedure as the primary method of evaluating the axilla in most cases of early stage breast cancer. Because SLNB has lower rate of morbidity and lower false positive rate than ALND. Historically, there was a presumed therapeutic benefit derived from ALND .It was a component of the radical mastectomy procedure that was necessary to treat breast cancer. No study has ever shown a survival benefit from ALND. (19)

In current clinical practice, most patients with breast cancer and palpable axillary nodes or nodal metastases identified by imaging(US-FNA) before surgery (“clinically node-positive” patients) will undergo ALND without performing SLNB. “Clinically node-negative” patients with T1 or T2 tumors undergo SLNB. If SLNB results are negative, no further axillary dissection is done because there is no survival benefit in these patients. But either people with positive SLNB or people clinically node-positive identify by US-FNA, go through an ALND.(5)

Figure1: Possible algorithm for axillary staging in patients with early stage invasive breast cancer. The +/− indicates that in the setting of palpable lymph nodes, US evaluation and US-FNA or US-CNB may or may not be used.



Resource:-*RadioGraphics, http://pubs.rsna.org/doi/abs/10.1148/rg.336125060 Published in: Jacob S. Ecanow; Hiroyuki Abe; Gillian M. Newstead; David B. Ecanow; Jan M. Jeske; RadioGraphics 2013, 33, 1589-1612*.

The recently published results of the American College of Surgeons Oncology Group(ACOSOG) Z0011 trial suggest that there may be no survival benefit for performing ALND in some patients with limited disease(diagnosed by SLNB). Although the results of this trial are currently being debated, the implication is that, ALND may not be necessary in some patients with limited axillary metastases.(20)(21)

Since publication of the results of the ACOSOG Z0011 trial, the indications for ALND are revised. This way the procedure(ALND) is restricted to patients with more advanced disease. In conclusion is important to identify those patients who need axillary dissection, avoiding ALND in false-positive results. So a lot of unnecessary ALND are being spared. In consequence the morbidity is being reduced for these patients.

According to the current clinical practice the axillary clearance should be performed in patients with axillary nodes metastasis proved by a fine needle aspiration guided by ultrasounds(US-FNA) or patients who had no suspicion lymph node metastasis but has a positive SLNB. However some patients with positive SLNB that meet Z0011's criteria can be treated without ALND. (22)(23)(20)

The ZOO11's criteria are (20):

-T1- T2 tumors

-with 1-2 positive SLN

-undergoing breast conservative surgery

-who can undergo radiotherapy after the surgery

This is a great advance due to the complications of the axillary surgery:

Amongst the acute, the recognized side effects are axillary web syndrome (AWS), seroma, wound infection, decreased range of motion (ROM) of the ipsilateral shoulder, and change in sensation (paresthesia).(24)

Long-term side effects may overlap with the acute morbidities and are comprised of such toxicities as lymphedema, decreased ROM of the ipsilateral shoulder, and paresthesia.(24)

The aim of this study is identify those patients with clinically node positive by FNA-US who are unnecessary undergoing an ALND.

TO SUM UP : The axillary lymph node dissection(ALND) is an aggressive technique to reduce risk of recurrence of cancer. It is already proved that in some selected patients

ALND doesn't change either survival and recurrence rates (ACOSOG Z0011 trial). But it is only studied in people clinically node-negative with a positive sentinel lymph node. And what about people with a positive fine needle aspiration guided by ultrasound that meet the same criteria with a limited lymph node disease?

The aim of this study is to demonstrate that results of Fine Needle Aspiration guided by ultrasound identify same lymph node that the Sentinel Lymph Node Biopsy. That would mean that a positive US-FNA isn't an absolute indication of ALND. This would be an important finding because could change the algorithms of axillary treatment in breast cancer. Saving this kind of procedure (ALND) will highly reduce the morbidity of surgery proceedings and complications. It will also reduce the costs of the breast surgeries and the surgical time.

3.2 JUSTIFICATION

Breast cancer is the most common cancer among women worldwide. That makes an important issue the management of this tumor. Besides regional lymph node status remains one of the strongest predictors of long-term prognosis on this cancer. Nowadays there is a controversy in which is the suitable treatment of the axillary affection.

Years ago, before 2010, people who had positive Sentinel Lymph Node Biopsy (SLNB) underwent an Axillary Lymph Node Dissection (ALND). Today thanks to the ACOSOG Z0011 Trial, it is accepted that patients who meet criteria of this study doesn't benefit from the ALND. The American College of Surgeons Oncology Group trial Z0011 was a prospective, randomized, multicenter trial comparing overall survival between patients with positive sentinel lymph nodes (SLNs) who did and did not undergo axillary lymph node dissection (ALND). The recurrences of local lymph node and the survival over the two groups of patients were observed(21). It wasn't found any significant differences.

The Z0011 criteria were:

- Tumors smaller than 5 cm (T1-T2)
- Negative clinical lymph node
- 1 or 2 sentinel lymph node(by SLNB) affected
- Undergoing a conservative breast surgery
- Patients who could receive radiotherapy optimized for the axilla

This study has changed the paradigm of the axillary metastases, reducing the amount of ALND without sacrificing survival or local control.

This is a great advance due to the complications of the axillary surgery (ALND). They can be acute and chronic complications(24). Amongst the acute, the recognized side effects are:

- Axillary web syndrome (AWS) or cording
- Seroma
- Wound infection
- Decreased range of motion (ROM) of the ipsilateral shoulder and change in sensation (paresthesia)

Long-term side effects may overlap with the acute morbidities and are summed up as: toxicities as lymphedema, decreased ROM of the ipsilateral shoulder, and paresthesia.

Another advantage of avoiding the Lymph Node Clearance is the reduction of the hospital´s costs(22).

My study focuses on one group of patients that is not represented in the ACOSOG Z0011 Trial: Patients diagnosed of limited primary breast cancer with positive Fine Needle Aspiration (US-FNA). This subgroup of patients nowadays is all treated with ALND according with the protocol. And the question is: Does all US-FNA positive tumors really benefit of the Axillary Lymph Node Dissection? Or are we over treating this group of patients?(25)

To answer these questions, first of all we want to prove if the results of Ultrasound guided Fine Needle Aspiration (US-FNA) are the same as the results of the Sentinel Lymph Node Biopsy (SLNB) in patients with tumor's characteristics similar to the included in Z0011 trial.

The aim of the study is to evaluate the sensitivity of the US-FNA to detect the sentinel lymph node. That would mean that people with a positive US-FNA that meet Z0011 criteria could avoid undergoing the Axillary Lymph Node Dissection. Because these patients with the same lymph node findings by SLNB would avoid ALND.

The study would carry out by inserting a clip in the lymph node aspirated in the US-FNA. Then during the surgery we would undergo the SLNB. We will check if the positive lymph node aspirated is the same positive sentinel lymph node. In the study the ALND is going to be done to patients with positive US-FNA as it is done according to the current clinical practice. Not doing the ALND in patients that it's indicated according to the protocol accepted wouldn't be ethical.

If the results are statistically significant it would suppose a great advance. On one hand, it would reduce the morbidity for those patients who avoid the ALND. On the other hand, it would suppose a way of saving money, because all these surgeries would be shorter and cheaper if the ALND is avoided.

4. BIBLIOGRAPHY

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J cancer [Internet]. 2015 Mar 1 [cited 2017 Jan 22];136(5):E359-86. Available from: http://doi.wiley.com/10.1002/ijc.29210

2. J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman FB. Fact Sheets by Population [Internet]. Available from: http://globocan.iarc.fr/Pages/fact_sheets_population.aspx

3. Sociedad Española de Oncología Medica(SEOM. Las Cifras del Cáncer en España 2016.

4. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Breast cancer statistics | World Cancer Research Fund International [Internet]. Available from: http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/breast-cancer-statistics

5. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.

6. Marcos-Gragera R, Loreto M, Gil V, Pujolràs MB, Fuentes Fernández J. CanGir 2007-9 Proyeccions de la incidència,2013-2014. 2013;

7. Oeffinger KC, Fontham ETH, Etzioni R, Herzig A, Michaelson JS, Shih Y-CT, et al. Breast Cancer Screening for Women at Average Risk. JAMA [Internet]. 2015 Oct 20 [cited 2017 Jan 23];314(15):1599. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2015.12783

8. Lonning PE. Breast cancer prognostication and prediction: are we making progress? Ann Oncol [Internet]. 2007 Sep 1 [cited 2017 Jan 23];18(Supplement 8):viii3-viii7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17890212

9. Fu L, Liu M, Yu X, Li X. [Expressions of epithelial cell adhesion molecule, vimentin and N-cadherin in molecular subtypes of breast cancer and the correlation among them]. Zhong Nan Da Xue Xue Bao Yi Xue Ban [Internet]. 2016 Nov 28 [cited 2017 Jan 23];41(11):1137–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27932757

10. Acosta-Martínez M, Karchmer-Krivitzky S, Melgar-Barriga G, Molinar-Horcasitas ML, Garza-Arrieta J. [Mammographic and ultrasonographic BIRADS classification and its correlation with histopathologic findings]. Ginecol Obstet Mex [Internet]. 2016 Mar [cited 2017 Jan 23];84(3):136–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27424439

11. Fisher B, Anderson S, Bryant J, Margolese RG, Deutsch M, Fisher ER, et al. Twenty-Year Follow-up of a Randomized Trial Comparing Total Mastectomy,

Lumpectomy, and Lumpectomy plus Irradiation for the Treatment of Invasive Breast Cancer. N Engl J Med [Internet]. 2002 Oct 17 [cited 2017 Jan 23];347(16):1233–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12393820

12. van Dongen JA, Voogd AC, Fentiman IS, Legrand C, Sylvester RJ, Tong D, et al. Long-term results of a randomized trial comparing breast-conserving therapy with mastectomy: European Organization for Research and Treatment of Cancer 10801 trial. J Natl Cancer Inst [Internet]. 2000 Jul 19 [cited 2017 Jan 23];92(14):1143–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10904087

13. Bundred NJ, Morgan DAL, Dixon JM. ABC of Breast Diseases: Management of regional nodes in breast cancer. BMJ. 1994;309(6963).

14. Vijayaraghavan GR, Vedantham S, Kataoka M, DeBenedectis C, Quinlan RM. The Relevance of Ultrasound Imaging of Suspicious Axillary Lymph Nodes and Fine-needle Aspiration Biopsy in the Post-ACOSOG Z11 Era in Early Breast Cancer. Acad Radiol [Internet]. 2016 Dec 1 [cited 2017 Jan 23]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/27916595

15. Abe H, Schmidt RA, Kulkarni K, Sennett CA, Mueller JS, Newstead GM. Axillary Lymph Nodes Suspicious for Breast Cancer Metastasis: Sampling with US-guided 14-Gauge Core-Needle Biopsy—Clinical Experience in 100 Patients. Radiology [Internet]. 2009 Jan [cited 2017 Jan 17];250(1):41–9. Available from: http://pubs.rsna.org/doi/10.1148/radiol.2493071483

16. Ecanow JS, Abe H, Newstead GM, Ecanow DB, Jeske JM. Axillary Staging of Breast Cancer: What the Radiologist Should Know. RadioGraphics [Internet]. 2013 Oct [cited 2017 Jan 2];33(6):1589–612. Available from: http://pubs.rsna.org/doi/10.1148/rg.336125060

17. Ultrasonography of superficial lymph nodes: benign vs. malignant. Rev Med Ultrason. 2012;14(4):294–306.

18. Usmani S, Ahmed N, Saleh N Al, Huda F abu, Amanguno HG, Amir T, et al. The clinical utility of combining pre-operative axillary ultrasonography and fine needle aspiration cytology with radionuclide guided sentinel lymph node biopsy in breast cancer patients with palpable axillary lymph nodes. Eur J Radiol [Internet]. 2015 Dec [cited 2016 Dec 13];84(12):2515–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26474908

19. Boland MR, Prichard RS, Daskalova I, Lowery AJ, Evoy D, Geraghty J, et al. Axillary nodal burden in primary breast cancer patients with positive pre-operative ultrasound guided fine needle aspiration cytology: Management in the era of ACOSOG Z011. Eur J Surg Oncol [Internet]. 2015 Apr [cited 2016 Dec 13];41(4):559–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25648466

20. Acea Nebril B, García Novoa A, Garea CC, Villar RR, Freijanes PS, Martínez LC, et

al. Repercusión de los criterios ACOSOG Z0011 sobre la indicación de la linfadenectomía axilar y el control locorregional en mujeres con ganglio centinela metastásico. Resultados preliminares tras cuatro años de aplicación clínica. Rev Senol y Patol Mamar [Internet]. 2015 Jul [cited 2016 Dec 17];28(3):105–12. Available from: http://linkinghub.elsevier.com/retrieve/pii/S021415821500047X

21. Le VH, Brant KN, Blackhurst DW, Schammel CMG, Schammel DP, Cornett WR, et al. The impact of the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial: An institutional review. The Breast [Internet]. 2016 Oct [cited 2017 Jan 23];29:117–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27479042

22. Fillion MM, Glass KE, Hayek J, Wehr A, Phillips G, Terando A, et al. Healthcare Costs Reduced After Incorporating the Results of the American College of Surgeons Oncology Group Z0011 Trial into Clinical Practice. Breast J [Internet]. 2016 Nov 30 [cited 2017 Jan 23]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/27900818

23. Wernicke AG, Shamis M, Sidhu KK, Turner BC, Goltser Y, Khan I, et al. COMPLICATION RATES IN PATIENTS WITH NEGATVE AXILLARY NODES 10-YEARS AFTER LOCAL BREAST RADIOTHERAPY FOLLOWING EITHER SENTINEL LYMPH NODE DISSECTION OR AXILLARY CLEARANCE.

24. Nielsen HM, Friis RB, Linnet S, Offersen BV. Loco-regional morbidity after breast conservation and axillary lymph node dissection for early breast cancer with or without regional nodes radiotherapy, perspectives in modern breast cancer treatment: the Skagen Trial 1 is active. Acta Oncol (Madr) [Internet]. 2017 Jan 20 [cited 2017 Jan 23];1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28105873

25. Y.L. JT, McGowan K, Cooley G, McLaughlin R, Sugrue M. The role of ultrasound guided core biopsy of axillary nodes in predicting macrometastases and avoiding overtreatment outside ACOSOG Z0011 parameters. The Breast [Internet]. 2015 Feb [cited 2017 Jan 25];24(1):57–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25491190

26. Siew Ng C, Arulanantham S, Joon Khoo J, Sabaratnam S, Fong Lee Y, Fang Ngim C. Skill validation study on sentinel lymph node biopsy in breast cancer and the challenges of false-negative, in-transit and micrometastatic nodes. Med J Malaysia. 2016;71(5).

27. Chen SL, Iddings DM, Scheri RP, Bilchik AJ. Lymphatic mapping and sentinel node analysis: current concepts and applications. CA Cancer J Clin [Internet]. [cited 2017 Jan 25];56(5):292-309-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17005598

28. Szychta P, Westfal B, Maciejczyk R, Smolarz B, Romanowicz H, Krawczyk T, et al. Intraoperative diagnosis of sentinel lymph node metastases in breast cancer treatment with one-step nucleic acid amplification assay (OSNA). Arch Med Sci

[Internet]. 2016 Dec 1 [cited 2017 Jan 17];6(6):1239–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27904514

29. Vijayaraghavan GR, Vedantham S, Kataoka M, DeBenedectis C, Quinlan RM. The Relevance of Ultrasound Imaging of Suspicious Axillary Lymph Nodes and Fine-needle Aspiration Biopsy in the Post-ACOSOG Z11 Era in Early Breast Cancer. Acad Radiol [Internet]. 2016 Dec 1 [cited 2016 Dec 7]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/27916595

30. Gipponi M, Fregatti P, Garlaschi A, Murelli F, Margarino C, Depaoli F, et al. Axillary ultrasound and Fine-Needle Aspiration Cytology in the preoperative staging of axillary node metastasis in breast cancer patients. The Breast [Internet]. 2016 Dec [cited 2016 Nov 23];30:146–50. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0960977616301709

5. QUESTION

Is there an agreement between fine needle aspiration guided by ultrasounds (US-FNA) and the sentinel lymph node biopsy (SLNB) results? Both of them are diagnosis test to detect lymph node metastasis in staging of axillary affection in the study of breast cancer. It is an important topic because axillary metastasis is one of the most important prognosis factors in breast tumors.

6. HYPOTHESIS

Main hypothesis:

There is a high proportion of PPV between both diagnosis tests: Fine Needle Aspiration guided by ultrasound (US-FNA) and Sentinel Lymph Node Biopsy (SLNB) in axillary affection in breast cancer.

Secondary hypothesis

- There are some covariables that influence in the expected findings between the studied procedures.

7. OBJECTIVES

Main Objective

The goal of the present work is to determine the agreement between the results of two diagnosis tests of the axillary affection in breast cancer. The FNA guided by ultrasounds (US-FNA) and SLNB detect the lymph node metastasis in the study of the loco regional disease. They are used to do the staging and risk assessment in breast cancer. We aim to compare the results of US-FNA with the results of the sentinel lymph node biopsy and check if the positives lymph nodes detected by US-FNA are the same and equally positive lymph node by SLNB.

It's important to prove the high sensitivity of the US-FNA identifying the sentinel lymph node. This way we will be able to propose saving on proceeding to an Axillary Lymph Node Dissection (ALND) in positive US-FNA. As well as we do nowadays for selected patients when they have just one or two sentinel lymph node affected by SLNB. This way in these patients we can only do a radiotherapy treatment over the lymph nodes.

Secondary Objective

- Evaluate if the agreement between both tests is due to possible interactions of other covariables.

8. MATERIAL AND METHODS

8.1 STUDY DESIGN

A diagnostic test study with a cross-sectional design carried out in a tertiary referral hospital in Girona within the Breast Pathology Unit which integrates a multidisciplinary team formed by gynecologists, general surgeons, radiologists, pathologists, oncologists, nuclear physicians and radiotherapists.

8.2 PARTICIPANTS

The study population is based on women diagnosed with breast cancer in the Breast Pathology Unit at Hospital Universitari de Girona Dr.Josep Trueta. A core needle biopsy will be used for preoperative diagnosis of breast tumor. All patients will undergo preoperative evaluations using mammography(identify primary tumor), ultrasound(US) and Fine Needle Aspiration(FNA) of suspicious lymph node and magnetic resonance imaging(MRI) according to the established protocol.

8.3 INCLUSION CRITERIA

- Patients diagnosed with primary breast cancer smaller than 5 cm(T1-T2)
- Patients with a positive FNA guided by ultrasound in the loco regional staging
- Patients with a negative FNA guided by ultrasound who undergo a sentinel lymph node biopsy(SLNB)
- Positive hormone receptors breast tumors

8.4 EXCLUSION CRITERIA

- Selected breast cancer tributary to neoadjuvancia (inflammatory breast cancer, triple negative breast tumor)
- Patients with tumors bigger than 5 cm (T3,T4)

- Patients with a triple negative tumor (all the hormone receptors are negative: estrogen (ER), progesterone (PR) and HER-2)
- Patients with a positive HER-2 tumor without antibody treatment(such as Herceptin)
- Patients with previous axillary radiotherapy
- Patients with radiotherapy contraindications (such as pulmonary disease…)
- History of previous ipsilateral breast cancer
- Patients with sentinel lymph node biopsy contraindications (such as pregnancy, allergy to the radioactive substance injected in the peritumoral skin)

8.5 SAMPLE SELECTION

A consecutive non-probabilistic sampling will be performed. Including women diagnosed of breast cancer T1-T2 with a positive US-FNA lymph node or, negative by US-FNA but who undergo SLNB. The sample recruitment will take place in Hospital Universitari Dr.Josep Trueta throughout one year.

8.6 SAMPLE SIZE

In 2014, at the Breast Pathology Unit department of the Hospital Universitari de Girona Josep Trueta, 389 new cases of breast cancer were diagnosed. Aproximately 70% (291) of them have a tumor size smaller than 5 cm(T1, T2) and low risk (positive hormone receptors),which are included in our study.

Taking this data into account and using other studies as a reference, we will accept a 0,05 alpha risk and a beta risk below 0,2 in a bilateral contrast , where 153 subjects are needed to detect a difference equal to or greater than 0,1 units. It is assumed that the proportion in the reference group is 0,5 . As it is a cross-sectional study any drop-out rate is expected. We can maybe have into account a percentage of people who don't want to enter in our study, approximately 30%.

8.7 PRINCIPAL VARIABLE

The main variable in our study is: the axillary lymph node affection as positive or negative. The axillary treatment decision depends on this variable.

The diagnosis of the axillary disease is done with the complementary test performed according to the hospitals protocol to stage the axillar affection. These two techniques are: SLNB and US-FNA (see in section of procedures).

This variable will be measured as a nominal dichotomous qualitative variable: positive or negative.

8.8 COVARIABLES

These variables are included because we want to take them into account in our population.

-Obesity: >30 / ≤30, LNs are more readily identified by ultrasounds in thin people than obese. Obesity can interfere as well in access to LNs in SLNB. We are going to use the body mass index (BMI) in order to collect the data.

Obese: BMI>30; Not obese ≤30

-The age:

>70; < 70

-The tumor grade: the highest grade means more lymph node affected. (Annex II)

Grade 1; Grade 2; Grade 3

-Hormonal status:

ER+ and PR+; ER+ and PR-; ER- and PR+

-Tumor size:

T1; T2

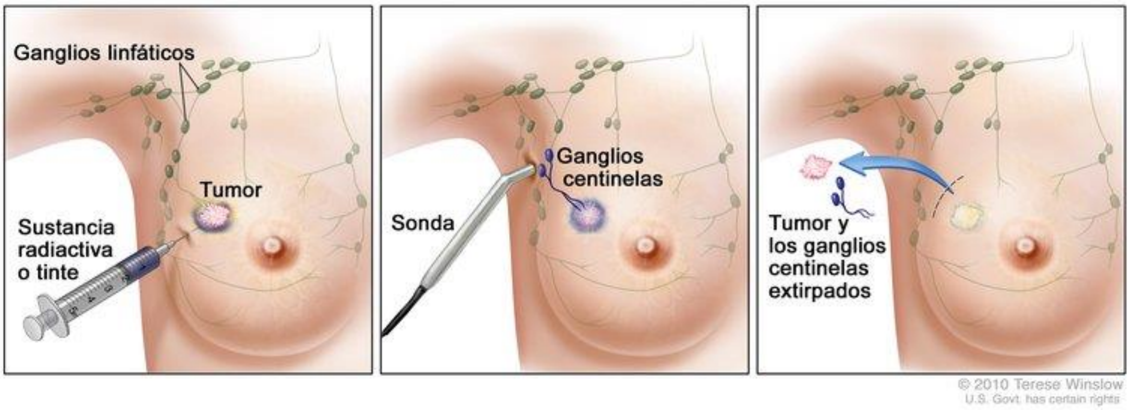
9. PROCEDURES

<u>9.1 Sentinel lymph node biopsy (SLNB)</u>

SLNB is a multidisciplinary procedure requiring close collaboration among nuclear medicine, surgery and pathology. According to the SLN hypothesis, tumor cells migrating from a primary tumor metastasize to one or a few lymph nodes (LNs) before involving other LNs. The test consists on an injection of radiolabeled colloid (Tc 99) into the skin and breast tissue around the area of the tumor or the subareolar area. That permits the identification of one or more SLNs in the majority of patients. The status of the sentinel nodes accurately predicts the status of the remaining axillary LNs. It is performed to identify those patients without axillary node involvement, who doesn't need a more morbid Axillary Lymph Node Dissection (ALND).

The technique consists on the injection of radioactive colloid into the skin of the breast (intradermal, subdermal) or parenchyma of the breast , usually near the tumor .These tracers then enter lymphatic channels, and passively flow to LNs. One or a few LNs are labeled, making it possible to identify those first receiving drainage from the tumor. The SLN can be variably located, but it'is usually in the low axilla (level1). With isotope, the surgeon uses a handheld gamma probe intraoperatively to find the axillary "hot spots" corresponding to the SLN. They remove hot nodes until the axillary background radiation count falls below a defined threshold level. These hot nodes are removed and send to pathology for pathologic examination. (26)

Figure 2: Sentinel lymph node biopsy technique.(27)



The pathologist analyses the sentinel lymph node by OSNA. It is an intraoperative molecular method for the detection of metastatic breast cancer LNs. It detects mRNA expression of the epithelial marker cytokeratin 19 (CK19). CK19 is normally absent in lymph nodes and its high level of expression is seen in the majority of breast cancer cells. The number of copies of mRNA for CK19 can be assessed with the one-step nucleic acid amplification test (OSNA). Depending on the CK19 mRNA copy number amplified in SLN, the results are expressed as micrometastasis, macrometastasis, no metastasis or isolated cells.(28)

These results can be quantitative (copy number) or qualitative, for our study we are going to use the variable like a qualitative one. Besides we are taking the SLNB as a dichotomous qualitative variable taking as positive: the macrometastasis and as negative: the rest of results. This is because the ALND is only done in the macrometastasis so this is what we are taking as positive. Although nowadays a positive macrometastasis doesn't always mean to undergo a ALND, according to Z0011 criteria.(28)

A sentinel lymph node with CK19 mRNA copy number/µl:

No diluid sample CK19 cuantitative results		Cualitative results	Treatment decision
	5000≤copy number	(++) Macrometastasis	ALND or RT (depends on the tumor and patient characteristics)
	250≤copy number<5000	(+) Micrometastasis	Axillary radiotherapy(RT)
	100≤copy number<250	(-)L Low expression CK19 (no relevant) (-) No metastasis	RT or nothing (depends on the tumor and patient characteristics)
	copy number<100	(-) No metastasis	No axillary treatment

(28)

In our study we would do the axillary lymph node dissection as it is indicated in the patients with positive FNA. We are going to check if the positive lymph node that we have marked in the US-FNA is the same as the positive sentinel lymph node. After that, according to the current clinical practice all the axillary nodes are going to be examined by the pathologist to verified how many and which are the metastatic lymph nodes.

9.2 Fine Needle Aspiration guided by ultrasounds

The Ultrasound Guided Fine Needle Aspiration (US-FNA) is used for preoperative staging of axillary lymph nodes in breast cancer. The US-FNA is performed by radiologists specialized in breast imaging. The axilla is scanned using high-resolution (12Hz electronically focused linear-array transducer) ultrasound equipment (HDI5000, Philips Healthcare Ultrasound).The radiologist is in charge of selecting the lymph nodes to aspirate. They are chosen according to some signs suspicious of metastasis. Suspicious features are: focal or diffuse cortical thickening (>3mm), the absence of the fatty hilum, non-hilar-cortical vascular flow, irregular and invasive margins and a rounded shape. If all the lymph nodes appeared similar or normal ,would choose the lowest in the axilla because is considered the most likely to be the sentinel node. In case a couple of nodes seem suspicious, they can be both aspirated.(15)(29)

US-Guided FNA procedure:

It is used a 22- intramuscular gauge needle attached to a syringe to obtain specimens for cytological examination. The radiologist tries to aspirate in the thickest portion of the cortex of the most suspicious lymph node. The specimen is placed in CytoLyt solution (Cytyc) and sent to pathology. In the laboratory it is processed and examined by the cytologist. The results are reported as 3 options:(30)

-Satisfactory for cytologic evaluation, negative for malignancy

-Satisfactory for cytologic evaluation, positive for malignancy

-Insufficient for diagnosis. It is considered as a negative result. The US-FNA could be repeated in case there would be any other suspicious lymph node.

In some other hospitals the procedure is under local anesthesia, it isn't in Hospital Josep Trueta. Besides in our study we need to mark the lymph node that we have aspirated. At the same time we puncture the node, a clip will be inserted. Thanks to this, we can quickly identify during the surgery the lymph node we have aspirated. We decided to insert a clip to all the aspirated lymph nodes .That attitude will reduce the possible mistake identifying the aspirated lymph node after the cytological results (the results takes a couple of days). As well as we don't need to puncture again to the patient.

10 METHODS OF DATA COLLECTION

For data collection, the surgeons of the Breast Pathology Unit will inform the rest of the members of the unit about the study that is being carried out. Special requirement will be asked:

-to pathology department what concerns the microscopic and macroscopic metastasis of the lymph node.

-to the nuclear medicine department who are in charge of doing the sentinel lymph node biopsy

-to radiologist to do the clinical staging of the axillary affection (cN) by US-FNA

Most of the data will be collected from the electronic medical records of the participating women and will be reflected in the study database. Homogeneity in data collection must be ensured.

In each recruited patient:

-Firstly, they will be informed about the study and will be asked to join the study. They will have to sign the consent form if they want to participate with the study. The characteristics of patient's tumor will be recorded in the data base

-Secondly, during the US-FNA, the radiologist has to specify how much lymph nodes are marked with a clip and aspirated. The specimen will be sent to the pathologist and the results will be written in the database of the study.

- Thirdly, the day before of the breast conservative surgery the nuclear medicine physician will inject the colloid drug (TC99). The surgeons, helped by the nuclear medicine physician, will find the sentinel lymph node and check if it is the same lymph node that the radiologist identifies by the ultrasound (we will know it thanks to the clip inserted during de US-FNA). After having the pathology results, it will be introduced in the study data base. According to the current protocol, the ALND will be done in patients with positive US-FNA.

-Finally, all the study variable and covariables will be taken into account and will be recorded in the database.

The information will be obtained from:

Radiologist report: The image techniques MRI, mammography and US will be used for obtaining information about tumor size, nodal status.

Interventionist radiologist: the amount of suspicious axillary lymph nodes through the image of the ultrasound, besides they have to mark with a clip them and do the ultrasound guided FNA that will be taken to Pathology.

Pathologist report: the needle core biopsy of the tumor will provide information about histological type, grade and tumor biology (receptor expression and molecular type). Additionally, the results of the analysis of the lymph nodes which is what we are studying in our project. Either from the FNA, as well as the SLNB.

Surgeon report: intraoperative identification of the SLN and check if are the same that we marked in the ultrasound guided FNA before sending all the lymph node extracted to pathologist.

This table summarizes the data collection process

Informant	Data Resource	Prior to Surgery	During Surgery	After Surgery
Radiologist	MRI Mammography	Tumor size, microcalcification presence, multicentric lesions.		
Interventional Radiologist	Ultrasounds US- Fine Needle Aspiration + clip the suspicious LN	Clinical status of lymph node (cN)		
Pathologist	Needle Core Biopsy of the tumor	Histological type , grade and tumor biology and hormones receptors		
	FNA of lymph node	Affection of the axillary lymph nodes aspirated(by US-FNA)		
	Sentinel Lymph Node Biopsy		Histology(micro or macro metastasis) of sentinel lymph node	
Surgeon			Check if lymph node with a clip are the same as the sentinel ones	
Nuclear Medicine Doctor	Sentinel Lymph Node Biopsy		Identify the SLN by handed gamma probe (device that detects radioactivity)	

11 STATISTICAL ANALYSIS

11.1 UNIVARIATE ANALYSIS

Our study is based on a descriptive analysis of the sample's and the test's results characteristics. The univariate or descriptive analysis results will be expressed as percentages for categorical variables. Quantitative continuous variables will be described by means. It will be used parametric or not parametric test depending on normal distribution of the variables.

11.2 BIVARIATE ANALYSIS

We want to estimate proportions of positive predictive value between FNA-US and SLNB and if we obtain the same results why in some patients is indicated avoiding ALND and in the other group is not? Evaluate the validity of the diagnostic test based on the sensitivity an positive predict value(PPV) of the ultrasound guided fine needle aspiration to identify the sentinel lymph node what means that have the same result. We will evaluate as well the specificity and negative predict value (NPV). We are going to build a contingence table reflecting the true positives, the false positives, the true negatives and the false negatives to calculate sensitivity and specificity.

Both variables are qualitative dichotomies because you can have positive or negative as result of the diagnosis tests.

		SLNB	
		+	-
US-FNA	+	A	B
	-	C	D

Sensitivity = a/(a+c)

Specificity = d/(b+d)

PPV = a/(a+b)

NPV = d/(c+d)

11.3 STRATIFICATION OF THE RESULTS DEPENDING ON COVARIABLES

Having the results of this data we can stratify the results by the covariables with the aim of detecting any interaction that may affect the conclusions of the study.

We can stratify the results depending on the obesity because is a covariable that can interfere in the accuracy of the ultrasounds to diagnose. Obesity (yes / no)

As well as we can stratify the rest of covariables previously explain: age (<70/>70), tumor grade (grade 1, grade 2 , grade 3), hormone status (PR and ER positive/ positive PR and negative ER/ negative PR and positive ER), tumor size (T1/T2)

A *p* value of less than 0.05 will be used to determine statistical significance.

12 ETHICAL ASPECTS

Right of non-maleficence is not affected in our study. The only proceeding that we are including in positive US-FNA patients which is not done in the current clinical practice is injection of the radioactive colloid in the tumor. It is a procedure of the SLNB, which is not necessary in patients with a positive US-FNA. But this injection has a very low morbidity (skin allergic reaction). In addition people, who have contraindications for the radioactive colloid, are excluded from the study.

This study will be evaluated by the Clinical Research Ethical Committee (CEIC, Comitè d'Ètica d'Investigació Clínica) of Hospital Universitari Dr. Josep Trueta. It will assess if the study fulfils the required criteria for being approved; moreover, the recommendations given by the committee will be taken into account.

Related to the participants, they will be given the participant information sheet (see Annex IV) and they will be asked to sign the informed consent in order to be included in the study (see Annex V).

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.

Confidential rules will be respected and all study participants will be informed according to article 5 from Spanish Organic Law 15/1999, de 13 de diciembre, de Regulación del Tratamiento Automatizado de los Datos de Carácter Personal. The right of accessing to any kind of information concerning the patient is guaranteed as well as the participants' right of consulting, modifying or erasing the personal data from their personal file. All data will be managed anonymously.

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

13 LIMITATIONS OF THE STUDY

In the study we have to acknowledge the limitations that could interfere in the results. The most important ones are:

- Due to the fact that Ultrasounds is an operator-dependent technique, we need a well-trained radiologist in order to have the most possible accuracy. It is going to be carried out by the same radiologist all the US-FNA so we are not including the differences between operators into the covariables that affect to our study. But this can affect to the external validity of the study because all the ultrasounds are being carry out by the same person. To solve this problem in future studies, US-FNA should be done by more than one radiologist in order to evaluate the degree of concordance between the radiologists. So they should use the inter-operator variability as a covariable to have into account in future studies.
- SLNB technique is an operator-dependent technique and requires trained nuclear medicine physicians, breast pathology surgeons and pathologists. The SLNB is a procedure that has been carried out in our hospital for many years. What means that our multidisciplinary team is really good trained for these proceedings. This can affect to the external validity of the study. So after this study, a multicentric design must be carried out to analyze the variability on the results between our hospital and other institutions.
- The false negative of both: US-FNA and SLNB, are a limitation that we are assuming. However these rates are really low and are no statistically significant. In fact, in the current clinical practice this risk is already assumed.
- The results will be from a single institution; as a consequence, the results might not be generalized to the general population or to other institutions. Additional research will be required to confirm our findings as a multicentric study.
- Maybe with the exclusion criteria we are missing information. But our exclusion criteria are not very restrictive so we are not going to have a huge problem of external validity in this part of the study.
- We don’t have a detection bias because in patients that we know is positive the US-FNA the pathology analysis of the sentinel lymph node is done by a machine (OSNA method).

14 WORK PLAN

Investigators: Elisabet Barahona(EB), Ester Vila(EV), Francesc Tuca(FT), Alexandra Bonmatí(AB).

Collaborators: radiologists, pathologists, nuclear medicine physicians

The sequence of activities carried out by the research team is gathered in 5 phases:

The research protocol is going to be evaluated by the hospital's ethical committee before starting, in order to receive their authorization to perform the study proposed.

1. COORDINATION PHASE (3 months)

   It will involve all the investigators and collaborators.

   The research protocol will be developed through a detailed and proper definition of the procedures that will be carried out in our study. At the beginning of the study, an organizational meeting for the research team will be performed. The study chronogram will be show and the data collections circuits will be specify and set up. We will ensure that all the research investigators and collaborators agree with the protocol. We will accept and have into account any suggestion, problem identification and evaluation of the research protocol.

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

2. FIELD WORK (24 months)

   -Pick the patients: During a recruitment period of 1 year a detailed work of sampling will be carried out meeting inclusion and exclusion criteria already specified.

   -Fine Needle Aspiration guided by Ultrasounds will be carried out to every patient. The specimen will be examined by the pathologist.

   -Sentinel Lymph Node Biopsy during the surgery will be done. The specimen will be examined by the pathologist.

   -Axillary Lymph Node Dissection will be carried out to the patients in whom it is indicated according to the current protocol.

3. DATA EXTRACTION AND PROCESSING DATABASE(6 months)

   It is required for this part of the study the statistical support.

Data will be entered in the database by the statistician every 6 months, alongside with the periodic data quality control in order to analyze all the collected data. Regularly, an analysis of data will be performed in order to control its evolution.

4. DATA ANALYSIS(3 months)

   After processing the database, the data collected will be analyzed using the appropriate statistical test according with the statistician. Firstly, a descriptive, sensitivity and specificity test, secondary, a stratification by covariables will be performed.

5. INTERPRETATION OF THE RESULTS(2 months)

   -Investigators and collaborators

   -A results interpretation will be performed and the corresponding articles will be written.

6. PUBLICATION OF THE RESULTS (1month)

   -Investigators and collaborators

   -Write and edit the articles to publish them.

TIME SCHEDULE

The proposed work plan has been divided into 5 phases. The schedule of project tasks is shown below:

Phase	Personal	2017			2018		
		Apr-Jun	July-Sept	Oct-Dec	Jan-Mars	Apr-June	July-Sept
Coordination phase	All team						
Field work	EB, EV, FT, AB						
Data extraction and processing database	Statistical support						
Data analysis	Investigators and statistical support						
Interpretation, publication and dissemination of the results	Investigators and statistical support						

15 FEASIBILITY

This project will take place at Hospital Universitari Dr. Josep Trueta in Girona, where the hospital itself will provide all means for developing the study: operating rooms, handheld gamma probe, ultrasound, fine needle to aspirate, clips and pathologist department availability. All the material we need is available every day in this hospital because both diagnosis tests are procedures are usually done in the daily routine in this hospital.

Additionally, the hospital will provide the informatics equipment suitable for processing the database for the study development without additional cost.

As our team does not habitually have specialized professionals in statistics we will hire a statistician to do the statistical analysis.

The supplementary costs include:

-The radioactive colloid injection in the patients with positive US-FNA

-The use of the handheld probe during the sentinel lymph node biopsy in patients with positive US-FNA (material available in the hospital).

-The statistician

16 BUDGET

In the feasibility section is described the means available. As we have said in the work plan we will hire a statistician to do statistical analysis.

Either the US-FNA as the SLNB and the pathologist procedures are all included in the National Health Service provisions and are common procedures which doesn't mean additional health cost. The extra costs are the SLNB in positive US-FNA patients.

		BUDGET PROPOSAL		
		Quantity	**Cost per unit**	**Cost**
Personnel costs	Statistician	1	35/h	1.680€
Goods and services	**Consumables**			
	-Radioactive colloid injection(for positive US-FNA)	77	150€	11.550€
Travel and subsistence arrangements	**Dissemination of the results**			
	Inscription to Congreso de la Sociedad Española de Senología y Patología Mamaria (SESPM)		600€	600€
	Costs of the trip -Flights -Accomodation -Subsistence allowance	 2 2 2	 130€ 170€ 200€	 260€ 340€ 400€
Publication	Publication. Open access article fees	1	1500€	1500€
TOTAL				16.330€

17 CLINICAL AND HEALTHCARE IMPACT

Despite the fact that breast cancer has been studied much, there isn't a total agreement in some therapeutic decisions. Nowadays doctors try to be less aggressive in the treatments and perform more conservative surgeries, avoiding no necessary morbidities. That's why our study proposes a way of saving aggressive surgeries which is the axillary lymph node dissection (ALND). That would mean to avoid ALND complications such as: axillary web syndrome (AWS), seroma, wound infection, decreased range of motion (ROM) of the ipsilateral shoulder, and change in sensation (paresthesia). In addition it would reduce the surgical time.

Years ago the protocols of the current clinical practice were even less conservative, and an ALND was done to every breast cancer diagnosed. The radical mastectomy was performed within the ALND. After, SLNB was discovered and used to minimize the amount of axillary surgeries. Then recently it was demonstrated that even if you have one or two sentinel lymph node affected, you don't need to undergo an axillary clearance according to Z0011 Trial.

What we want to demonstrate in our study is the sensitivity of US-FNA to detect the sentinel lymph node, and evaluate the agreement between SLNB and US-FNA results. What means that the lymph nodes "clinically positive" diagnosed by US-FNA are the same detected by the sentinel lymph node biopsy so it doesn't suppose a further axillary disease.

This study pretends to work as a tool and a resource for the healthcare staff to take better and more conservative decisions for the treatment of the patient. At the same time it can entails a way of saving money and reducing the surgical time.

If the results are relevant and our hypothesis is validated, it would change the treatment procedure in patients with a positive result from US-FNA.

Then, with the results obtained in the study, a woman who would previously undergo an axillary dissection won't need it now. So women with small tumors, low grade, and positive hormone receptors won't always need the ALND.

This study should be seen as a basis for future research: further studies focused in applying the research to clinical trials. As well as doing the same study in other hospitals in order to do a multicentric study and have more external validity. This would allow the extrapolation of the results for the general population and would confirm our findings.

18 ANNEXES

18.1 Annex I. HISTOLOGY AND STAGING

- The main histopathological breast cancer types are the following:

IN SITU CARCINOMAS	INVASIVE CARCINOMAS
-NOS(not otherwise specified) -Intraductal (DCIS, LCIS) -Paget's disease and intraductal	-NOS -Ductal -Inflammatory -Medullary, NOS -Medullary with lymphoid stroma -Mucinous -Papillary(predominantly micropapillary pattern -Tubular -Lobular -Paget's disease and infiltrating -Undifferentiated -Squamous cell -Adenoid cystic -Secretory -Cribriform

Adapted from:

- Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, van de Vijver MJ. WHO Classification of Tumors of the Breast. 4th ed. Lyon: IARC; 2012.

- Breast cancer staging. Definition of the TNM categories.

TNM: Tumor – node – metastases. Staging system for carcinoma of the breast.

Primary tumour (T)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma *in situ*
Tis(DCIS)	Ductal carcinoma *in situ*
Tis(LCIS)	Lobular carcinoma *in situ*
Tis (Paget's)	Paget's disease (Paget disease) of the nipple NOT associated with invasive carcinoma and/or carcinoma *in situ* (DCIS and/or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Paget's disease are categorised based on the size and characteristics of the parenchymal disease, although the presence of Paget's disease should still be noted.
T1	Tumour ≤20 mm in greatest dimension
T1mi	Tumour ≤1 mm in greatest dimension
T1a	Tumour >1 mm but ≤5 mm in greatest dimension
T1b	Tumour >5 mm but ≤10 mm in greatest dimension
T1c	Tumour >10 mm but ≤20 mm in greatest dimension
T2	Tumour >20 mm but ≤50 mm in greatest dimension
T3	Tumour >50 mm in greatest dimension
T4	Tumour of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules)[e]
T4a	Extension to the chest wall, not including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or oedema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma[f]
Regional lymph nodes (N) Clinical (cN)	
NX	Regional lymph nodes cannot be assessed (e.g. previously removed)
N0	No regional lymph node metastases
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[k] ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
N2a	Metastases in ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastases only in clinically detected[k] ipsilateral internal mammary nodes and in the absence of clinically evident level I, II axillary lymph node metastases
N3	Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with

	or without level I, II axillary lymph node involvement; or in clinically detected[k] ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastases in ipsilateral infraclavicular lymph node(s)
N3b	Metastases in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastases in ipsilateral supraclavicular lymph node(s)
Regional lymph nodes (N) Pathological (pN)	
pNX	Regional lymph nodes cannot be assessed (e.g. previously removed or not removed for pathological study)
pN0	No regional lymph node metastasis identified histologically
pN0(i−)	No regional lymph node metastases histologically, negative immunohistochemistry (IHC)
pN0(i+)	Malignant cells in regional lymph node(s) not >0.2 mm [detected by haematoxylin and eosin (H&E) staining or IHC including isolated tumour cell clusters (ITCs)]
pN0(mol−)	No regional lymph node metastases histologically, negative molecular findings (RT-PCR)
pN0(mol+)	Positive molecular findings (RT-PCR), but no regional lymph node metastases detected by histology or IHC
pN1	Micrometastases; or metastases in one to three axillary lymph nodes; and/or in internal mammary nodes with metastases detected by SLNB but not clinically detected[l]
pN1mi	Micrometastases (>0.2 mm and/or >200 cells, but none >2.0 mm)
pN1a	Metastases in one to three axillary lymph nodes, at least one metastasis >2.0 mm
pN1b	Metastases in internal mammary nodes with micrometastases or macrometastases detected by SLNB but not clinically detected[l]
pN1c	Metastases in one to three axillary lymph nodes and in internal mammary lymph nodes with micrometastases or macrometastases detected by SLNB but not clinically detected[l]
pN2	Metastases in four to nine axillary lymph nodes; or in clinically detected[k] internal mammary lymph nodes in the absence of axillary lymph node metastases
pN2a	Metastases in four to nine axillary lymph nodes (at least one tumour deposit >2.0 mm)
pN2b	Metastases in clinically detected internal mammary lymph nodes in the absence of axillary lymph node metastases
pN3	Metastases in ≥10 axillary lymph nodes; or in infraclavicular (level III axillary) lymph nodes; or in clinically detected ipsilateral internal mammary lymph nodes in the presence of one or more positive level I, II axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary

	lymph nodes with micrometastases or macrometastases detected by SLNB but not clinically detected ; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastases in ≥10 axillary lymph nodes (at least one tumor deposit >2.0 mm); or metastases to the infraclavicular (level III axillary lymph) nodes
pN3b	Metastases in clinically detected ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary lymph nodes with micrometastases or macrometastases detected by SLNB but not clinically detected[l]
pN3c	Metastases in ipsilateral supraclavicular lymph nodes
Distant metastasis (M)	
M0	No clinical or radiographic evidence of distant metastases
cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumour cells in circulating blood, bone marrow or other non-regional nodal tissue that are not >0.2 mm in a patient without symptoms or signs of metastases
M1	Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven >0.2 mm

➢ Stage grouping system for carcinoma of the breast.

Stage 0		
Tis	N0	M0
Stage IA		
T1	N0	M0
Stage IB		
T0	N1mi	M0
T1	N1mi	M0
Stage IIA		
T0	N1	M0
T1	N1	M0
T2	N0	M0
Stage IIIB		
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 IV		
Any T	Any N	M1

Reference:

- Breast. In: Edge SB, Byrd DR, Compton CC, et al., eds.: AJCC Cancer Staging Manual. 7th ed. New York: Springer, 2010, pp 347-76

18.2 Annex II. HISTOLOGICAL GRADE

The histological grade is determined by the Nottingham Histologic Score System (the Elston-Ellis modification of Scarff-Bloom-Richardson grading system)

The grade of a tumor is determined by assessing morphologic features: **(1)** tubule formation**, (2)** nuclear pleomorphism and **(3)** mitotic count.

1. Glandular (Acinar) / Tubular Differentiation

Score 1	**>**75% of tumour area forming glandular/tubular structures
Score 2	10% to 75% of tumour area forming glandular/tubular structures
Score 3	<10% of tumour area forming glandular/tubular structures

2. Nuclear pleomorphism

Score 1	Nuclei small with little increase in size in comparison with normal breast epithelial cells, regular outlines, uniform nuclear chromatin, little variation in size
Score 2	Cells larger than normal with open vesicular nuclei, visible nucleoli, and moderate variability in both size and shape
Score 3	Vesicular nuclei, often with prominent nucleoli, exhibiting marked variation in size and shape, occasionally with very large and bizarre forms

3. Mitotic Count (using a high power field diameter of 0.50 mm)

Score 1	Less than or equal to 7 mitoses per 10 high power fields
Score 2	8-14 mitoses per 10 high power fields
Score 3	Equal to or greater than 15 mitoses per 10 high power fields

Each of these features is assessed with a value of 1 (favourable) to 3 (unfavourable), and the scores for all three categories are added together. A combine score of 3-5 points is designated as grade 1; a combined score of 6-7 points is grade 2; a combined score of 8-9 points is grade 3.

Gx	Grade cannot be assessed
G1	Low combined histological grade (favourable)
G2	Intermediate combined histological grade (moderately favourable
G3	High combined histological grade (unfavourable)

Reference:

Breast Cancer and Breast Pathology [Internet]. Baltimore: Johns Hopkins University, Faculty of Medicine; 2012 [cited 2014 Oct 25]. Available from: http://pathology.jhu.edu/breast/grade.php

18.3 Annex III: BI-RADS CLASSIFICATION OF BREAST LESSIONS

ACR BI-RADS® Atlas Fifth Edition

QUICK REFERENCE

ULTRASOUND

Tissue composition *(screening only)*	a. Homogeneous background echotexture – fat b. Homogeneous background echotexture – fibroglandular c. Heterogeneous background echotexture	
Masses	Shape	Oval
		Round
		Irregular
	Orientation	Parallel
		Not parallel
	Margin	Circumscribed
		Not circumscribed - Indistinct - Angular - Microlobulated - Spiculated
	Echo pattern	Anechoic
		Hyperechoic
		Complex cystic and solid
		Hypoechoic
		Isoechoic
		Heterogeneous
	Posterior features	No posterior features
		Enhancement
		Shadowing
		Combined pattern
Calcifications	Calcifications in a mass	
	Calcifications outside of a mass	
	Intraductal calcifications	
Associated features	Architectural distortion	
	Duct changes	
	Skin changes	Skin thickening
		Skin retraction
	Edema	
	Vascularity	Absent
		Internal vascularity
		Vessels in rim
	Elasticity assessment	Soft
		Intermediate
		Hard
Special cases	Simple cyst	
	Clustered microcysts	
	Complicated cyst	
	Mass in or on skin	
	Foreign body including implants	
	Lymph nodes – intramammary	
	Lymph nodes – axillary	
	Vascular abnormalities	AVMs (arteriovenous malformations/ pseudoaneurysms)
		Mondor disease
	Postsurgical fluid collection	
	Fat necrosis	

MAMMOGRAPHY

Breast composition	a. The breasts are almost entirely fatty b. There are scattered areas of fibroglandular density c. The breasts are heterogeneously dense, which may obscure small masses d. The breasts are extremely dense, which lowers the sensitivity of mammography	
Masses	Shape	Oval
		Round
		Irregular
	Margin	Circumscribed
		Obscured
		Microlobulated
		Indistinct
		Spiculated
	Density	High density
		Equal density
		Low density
		Fat-containing
Calcifications	Typically benign	Skin
		Vascular
		Coarse or "popcorn-like"
		Large rod-like
		Round
		Rim
		Dystrophic
		Milk of calcium
		Suture
	Suspicious morphology	Amorphous
		Coarse heterogeneous
		Fine pleomorphic
		Fine linear or fine-linear branching
	Distribution	Diffuse
		Regional
		Grouped
		Linear
		Segmental
Architectural distortion		
Asymmetries	Asymmetry	
	Global asymmetry	
	Focal asymmetry	
	Developing asymmetry	
Intramammary lymph node		
Skin lesion		
Solitary dilated duct		
Associated features	Skin retraction	
	Nipple retraction	
	Skin thickening	
	Trabecular thickening	
	Axillary adenopathy	
	Architectural distortion	
	Calcifications	
Location of lesion	Laterality	
	Quadrant and clock face	
	Depth	
	Distance from the nipple	

MAGNETIC RESONANCE IMAGING

Amount of fibroglandular tissue (FGT)	a. Almost entirely fat b. Scattered fibroglandular tissue c. Heterogeneous fibroglandular tissue d. Extreme fibroglandular tissue	
Background parenchymal enhancement (BPE)	Level	Minimal
		Mild
		Moderate
		Marked
	Symmetric or asymmetric	Symmetric
		Asymmetric
Focus		
Masses	Shape	Oval
		Round
		Irregular
	Margin	Circumscribed
		Not circumscribed - Irregular - Spiculated
	Internal enhancement characteristics	Homogeneous
		Heterogeneous
		Rim enhancement
		Dark internal septations
Non-mass enhancement (NME)	Distribution	Focal
		Linear
		Segmental
		Regional
		Multiple regions
		Diffuse
	Internal enhancement patterns	Homogeneous
		Heterogeneous
		Clumped
		Clustered ring
Intramammary lymph node		
Skin lesion		
Non-enhancing findings	Ductal precontrast high signal on T1W	
	Cyst	
	Postoperative collections (hematoma/seroma)	
	Post-therapy skin thickening and trabecular thickening	
	Non-enhancing mass	
	Architectural distortion	
	Signal void from foreign bodies, clips, etc.	

Associated features	Nipple retraction	
	Nipple invasion	
	Skin retraction	
	Skin thickening	
	Skin invasion	Direct invasion
		Inflammatory cancer
	Axillary adenopathy	
	Pectoralis muscle invasion	
	Chest wall invasion	
	Architectural distortion	
Fat containing lesions	Lymph nodes	Normal
		Abnormal
	Fat necrosis	
	Hamartoma	
	Postoperative seroma/hematoma with fat	
Location of lesion	Location	
	Depth	
Kinetic curve assessment Signal intensity (SI)/time curve description	Initial phase	Slow
		Medium
		Fast
	Delayed phase	Persistent
		Plateau
		Washout
Implants	Implant material and lumen type	Saline
		Silicone - Intact - Ruptured
		Other implant material
		Lumen type - Single - Double - Other
	Implant location	Retroglandular
		Retropectoral
	Abnormal implant contour	Focal bulge
	Intracapsular silicone findings	Radial folds
		Subcapsular line
		Keyhole sign (teardrop, noose)
		Linguine sign
	Extracapsular silicone	Breast
		Lymph nodes
	Water droplets	
	Peri-implant fluid	

BI-RADS® ASSESSMENT CATEGORIES

Category 0:	**Mammography:** Incomplete – Need Additional Imaging Evaluation and/or Prior Mammograms for Comparison **Ultrasound & MRI:** Incomplete – Need Additional Imaging Evaluation	
Category 1:	Negative	
Category 2:	Benign	
Category 3:	Probably Benign	
Category 4:	Suspicious	Mammography & Ultrasound: Category 4A: Low suspicion for malignancy Category 4B: Moderate suspicion for malignancy Category 4C: High suspicion for malignancy
Category 5:	Highly Suggestive of Malignancy	
Category 6:	Known Biopsy-Proven Malignancy	

Resource: *ACR American College of Radiology :BI-RADS Atlas. Breast Imaging Reporting and Data System 2013. Authors: Carl J.D'Orsi,MD,Editor and Chair, Committee on BI-RADS; Edward A.Sickles, MD, Chair, Subcommittee on BI-RADS Mammography; Ellen B.Mendelson,MD,Chair, Subcommittee on BI-RADS Ultrasounds; Elizabeth A.Morris, MD, Chair, Subcommittee on BI-RADS MRI. Pp 10-11*

18.4 Annex IV. INFORMATION SHEET

HOJA DE INFORMACIÓN AL PACIENTE

Nombre del estudio:

SENSIBILIDAD Y ESPECIFICIDAD DE LA PUNCIÓN CON AGUJA FINA GUIADA POR ECOGRAFIA PARA DETECTAR EL GANGLIO CENTINELA. REPERCUSIÓN EN EL MANEJO TERAPEÚTICO DE LA AFECTACIÓN AXILAR EN EL CÁNCER DE MAMA.

Agradecemos su interés y colaboración en el estudio que estamos llevando a cabo desde la Unidad de Patología Mamaria (UPM) del Hospital Universitario Dr. Josep Trueta de Girona. A continuación, le explicamos en que consiste el estudio. El equipo a cargo del estudio le responderá a cualquier duda que le pueda surgir.

¿Cuál es el objetivo del estudio?

Este estudio tiene como principal objetivo evaluar la concordancia entre los resultados de dos pruebas diagnósticas utilizadas en la estadificación ganglionar del cáncer de mama. Esta estadificación es un aspecto importante ya que definirá el tratamiento a seguir. La comparación se realizara entre la punción con aguja fina guiada por ecografía y la biopsia selectiva del ganglio centinela.

Actualmente un paciente con un ganglio positivo detectado en la punción con aguja fina ya es tributario a vaciamiento ganglionar. Sin embargo en pacientes con las mismas características pero con ganglio centinela positivo no es necesaria dicha intervención. Este procedimiento quirúrgico conlleva más riesgo intraoperatorio además de posibles complicaciones tanto a largo como corto plazo. Lo cual representa una importante línea de investigación para evitar un sobre tratamiento en este perfil de pacientes.

¿Qué pasará si participo?

Usted seguirá el procedimiento diagnóstico y terapéutico habitual según el protocolo establecido por la Unidad de Patología Mamaria del Hospital Universitario Dr. Josep Trueta. Este estudio únicamente añadirá al protocolo establecido una inyección de un radiofármaco para desarrollar la técnica de la biopsia del ganglio centinela en caso de que el resultado de la punción con aguja fina guiada por ecografía resulte positivo. De esta manera este estudio no supondrá ningún procedimiento diagnóstico ni terapéutico adicional a parte de lo mencionado. Lo que se pretende es comparar los resultados de ambas pruebas diagnósticas que ya se realizan de rutina en nuestro centro.

De acuerdo con el objetivo de nuestro estudio, realizaremos ambas pruebas para la detección de afectación ganglionar. Durante la punción guiada por ecografía, se marcará el ganglio pinchado con un clip para reconocerlo durante la cirugía. Durante la cirugía se procederá al vaciamiento ganglionar axilar en los casos indicados según el protocolo actual vigente.

¿Es obligatorio participar?

La participación en el estudio es totalmente voluntaria. Si decide participar, se le pedirá que firme el consentimiento informado acorde a que usted ha entendido todo lo que implica participar en este estudio. Por el contrario, si decide no participar, eso no afectará ni modificará el manejo asistencial que ha de recibir.

¿Qué tengo que hacer para participar?

Para llevar a cabo este estudio y atendiendo a las disposiciones legales vigentes le solicitamos que rellene la siguiente autorización. Antes y después de firmar el consentimiento informado, puede preguntar cualquier duda a los médicos y personal sanitario responsable del estudio.

¿Mis datos se manejarán de forma confidencial?

Sí. La información recogida por este estudio será tratada según la Ley Orgánica de Protección de Datos de Carácter Personal (15/1999) según la cual, sus datos serán manejados de forma confidencial y solo serán utilizados con finalidad de investigación. Su identificación personal estará codificada a través de una serie numérica aleatorizada.

¿Qué pasará si cambio de opinión durante el desarrollo del estudio?

Si renuncia a seguir participando en el estudio una vez iniciado, no supondrá ningún castigo ni pérdida de beneficios para usted. Se le pedirá que siga los controles y seguimientos habituales.

¿Qué se hará con la información obtenida a partir del estudio?

Los resultados serán publicados en revistas de interés científico relacionadas con el área de conocimiento correspondiente a la patología mamaria para que otros centros y pacientes puedan aprovechar los resultados de nuestro estudio. Recuerde que todos sus datos de carácter personal son confidenciales y se utilizarán de forma anónima.

Muchas gracias por su atención,

Unidad de Patología Mamaria del Hospital Universitario Dr. Josep Trueta

18.5 Annex V. INFORMED CONSENT

CONSENTIMIENTO INFORMADO

Declaración del paciente,

Declaro que he estado correctamente informado por el miembro responsable del equipo investigador del presente estudio; sobre los objetivos del estudio así como sobre el proceso de selección de los datos personales; también declaro que he estado informado sobre el uso científico que se hará de mis datos personales, así como sobre el hecho de que la participación en el estudio es totalmente voluntaria, que puedo consultar sobre cualquier cuestión que considere oportuna y que puedo solicitar la retirada y eliminación de mi datos personales en cualquier momento del estudio. Además, he recibido una copia de este documento y la hoja informativa sobre el presente estudio.

Nombre, Apellidos y DNI del paciente

____

Firma: Fecha:

Apartado para la revocación del consentimiento informado

Yo,

____

con DNI________________________ revoco el consentimiento de participar en el estudio anteriormente mencionado.

Firma: Fecha:

18.6 Annex VI : INFORMED CONSENT FOR SENTINEL LYMPH NODE BIOPSY

CONSENTIMIENTO INFORMADO PARA BIOPSIA DEL GANGLIO CENTINELA

***¿DESEO SER INFORMADO** sobre mi enfermedad y la intervención que me van a realizar?

<table>
<tr><td colspan="4">DESEO QUE LA INFORMACIÓN de mi enfermedad y la intervención que me van a realizar le sea proporcionada a mi familiar / tutor / representante legal:</td></tr>
<tr><td>NOMBRE APELLIDOS (Paciente)</td><td>DNI</td><td>FIRMA</td><td>FECHA</td></tr>
<tr><td colspan="4">De acuerdo al art. 9 de la Ley 41/2002 de 14 de Noviembre “MANIFIESTO MI DESEO DE NO SER INFORMADO Y PRESTO MI CONSENTIMIENTO" para que se lleve a cabo el procedimiento descrito en este documento</td></tr>
<tr><td>NOMBRE APELLIDOS (Paciente)</td><td>DNI</td><td>FIRMA</td><td>FECHA</td></tr>
</table>

IDENTIFICACIÓN Y DESCRIPCIÓN DEL PROCEDIMIENTO
La operación consiste la extirpación de uno o más ganglios centinelas tras la localización del mismo mediante una sonda gamma detectora.
El ganglio centinela es el primer ganglio linfático que entra en contacto con las células cancerosas cuandoestas se diseminan desde el tumor primario. La localización mas habitual es la grasa de la axila. Mediante este procedimiento se pretende identificar la(s) zona(s) de drenaje del tumor mamario, localizar el (los) ganglio(s) centinelas y extirparlos de forma independiente para poder hacer un estudio selectivo de los mismos. De este análisis dependerá el tratamiento posterior. Si es factible se realizará un análisis intraoperatorio del ganglio y en caso de ser positivo se procederá a realizar la linfadenectomía. Si es negativo este primer estudio se esperará a la realización del protocolo
completo de ganglio centinela por parte del Servicio de Anatomía Patológica con el fin de saber si la linfadenectomía es necesaria.
También sé que cabe la posibilidad que durante la cirugía haya que realizar modificaciones del procedimiento por los hallazgos intraoperatorios para proporcionarme el tratamiento más adecuado.
El médico me ha advertido que el procedimiento requiere la administración de anestesia y que es posible que durante o después de la intervención sea necesaria la utilización de sangre y/o hemoderivados, de cuyos riesgos me informarán los servicios de anestesia y de hematología.
La realización de mi procedimiento puede ser filmado con fines científicos o didácticos, salvo que yo manifieste lo contrario. Toda esta información será tratada con la mayor confidencialidad ajustándose a lo dispuesto en la Ley Orgánica 15/1999, de 13 de diciembre de protección de datos de carácter personal.

OBJETIVOS DEL PROCEDIMIENTO Y BENEFICIOS QUE SE ESPERAN ALCANZAR
Mediante este procedimiento y como parte integral del tratamiento de mi cáncer de mama se pretende realizar una correcta estadificación de mi cáncer de mama mediante la realización de una biopsia del ganglio centinela.
ALTERNATIVAS RAZONABLES AL PROCEDIMIENTO
El médico me ha explicado que pueden existir tratamientos alternativos como la linfadenectomía axilar sin realización de la biopsia de ganglio centinela.
CONSECUENCIAS PREVISIBLES DE SU REALIZACIÓN
Esta intervención conlleva una cicatriz en la zona de la piel por encima de la localización del ganglio centinela.
CONSECUENCIAS PREVISIBLES DE SU NO REALIZACIÓN

RIESGOS FRECUENTES
Comprendo que a pesar de la adecuada elección de la técnica y de su correcta realización pueden presentarse efectos indeseables.
He sido informado de las posibles complicaciones que pueden suceder y en especial de las siguientes:
1. Infección de la herida quirúrgica.
2. Seroma.
3. Edema transitorio.
4. Cicatriz hipertrófica.
También me ha indicado la necesidad de advertir de mis posibles alergias medicamentosas, alteraciones de la coagulación, enfermedades cardiopulmonares, existencia de prótesis, marcapasos, medicaciones actuales o cualquier otra circunstancia.

RIESGOS POCO FRECUENTES, CUANDO SEAN DE ESPECIAL GRAVEDAD Y ESTÉN ASOCIADOS AL PROCEDIMIENTO POR CRITERIOS CIENTÍFICOS

RIESGOS Y CONSECUENCIAS EN FUNCIÓN DE LA SITUACIÓN CLÍNICA DEL PACIENTE Y DE SUS
CIRCUNSTANCIAS PERSONALES O PROFESIONALES
Por mi situación vital actual (diabetes, obesidad, hipertensión, anemia, edad avanzada...) puede aumentar la frecuencia o la gravedad de riesgos o complicaciones como:..
....
....
CONTRAINDICACIONES

PACIENTE			
D/Dª			
DECLARO que he comprendido adecuadamente la información que contiene este documento, que firmo el consentimiento para la realización del procedimiento que se describe en el mismo, que he recibido copia del mismo y que conozco que el consentimiento puede ser revocado por escrito en cualquier momento			
NOMBRE/APELLIDOS	**DNI**	**FIRMA**	**FECHA**

FAMILIAR / TUTOR / REPRESENTANTE			
D/Dª			
DECLARO que he comprendido adecuadamente la información que contiene este documento, que firmo el consentimiento para la realización del procedimiento que se describe en el mismo, que he recibido copia del mismo y que conozco que el consentimiento puede ser revocado por escrito en cualquier momento			
FAMILIAR / TUTOR / REPRESENTANTE	**DNI**	**FIRMA**	**FECHA**

MÉDICO RESPONSABLE			
DR D/Dª			
DECLARO que he comprendido adecuadamente la información que contiene este documento, que firmo el consentimiento para la realización del procedimiento que se describe en el mismo, que he recibido copia del mismo y que conozco que el consentimiento puede ser revocado por escrito en cualquier momento			
MÉDICO RESPONSABLE	**Nº COLEGIADO**	**FIRMA**	**FECHA**

18.7 Annex VII: INFORMED CONSENT FOR DIAGNOSIS/THERAPEUTIC PROCEDURES

CONSENTIMIENTO INFORMADO PARA PROCEDIMIENTOS DIAGNÓSTICOS/TERAPEÚTICOS

PACIENTE

Yo, _________________________ he leído la hoja informativa que me ha entregado el/la Dr/a ____________ . He comprendido las explicaciones que me ha facilitado, y el medico que me ha atendido me ha permitido todas las observaciones y me ha aclarado todas las dudas y preguntas que le he planteado. También comprendo que, en cualquier momento y sin necesidad de dar ninguna explicación, puedo revocar el consentimiento que ahora presto. Por eso, manifiesto que me considero satisfecho/a con la información recibida y que comprendo la indicación y los riesgos más frecuentes que pueden aparecer a nivel general durante la realización de este procedimiento diagnóstico/ terapéutico así como los riesgos concretos que pueden aparecer en mi caso dada la situación clínica y mis circunstancias personales (riesgos personalizados) y que son:

____

____

____

____

REPRESENTANTE LEGAL

Yo, ____________________________ en calidad de _____________________ del/ de la paciente ________________ he leído la hoja informativa que me ha entregado el/la Dr/a ______________.He comprendido las explicaciones que me ha facilitado, y el médico que me ha atendido me ha permitido todas las observaciones y me ha aclarado todas las dudas y preguntas que le he planteado. También comprendo que, en cualquier momento y sin necesidad de dar ninguna explicación, puedo revocar el consentimiento que ahora presto. En su presencia se ha dado al/ a la paciente toda la información pertinente adaptada a su nivel de entendimiento y está de acuerdo a someterse a este tratamiento/ procedimiento. Por eso, manifiesto que me considero satisfecho/a con la información recibida y que comprendo la indicación y los riesgos más frecuentes que pueden aparecer a nivel general durante la realización de este procedimiento diagnóstico/ terapéutico así como los riesgos concretos que pueden aparecer en el caso del/de la paciente dada su situación clínica y sus circunstancias personales (riesgos personalizados) y que son:

____

____

____

____

Y en tales condiciones doy mi consentimiento para que se practique el siguiente procedimiento:

Mamografía; Ecografía de mama; Mamografía proyecciones adicionales; BAG de mama guiada por ecografía; PAAF de ganglios axilares guiada por ecografía; Marcadores metálicos

Fecha: Firma del paciente y DNI

Firma del representante legal y DNI Firma del médico y nºcolegiado