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SELECTIVE EVALUATION OF CLIPPED NODES: IMPROVING AXILLARY EVALUATION AFTER NEOADJUVANT THERAPY IN NODE POSITIVE BREAST CANCER PATIENTS

Final Degree Project

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1. ABBREVIATIONS

ALND: Axillar Lymph Node Dissection

ANP: Aspiration Needle Puncture

CEIC: Clinical Research Ethics Committee

DFS: Disease free of survival

FN: False negative

FNR: False negative rate

LE: Lymphedema

MRI: Magnetic Resonance Imaging

OS: Overall survival

pCR: pathologic complete response

PET: Positron Emission Tomography

RC: Regional Control

REA: *Unitat de Reanimació Assistida*

SLN: Sentinel lymph node

SLNB: Sentinel lymph node biopsy

TNBC: Triple negative Breast Cancer

TP: True positive

US: Ultrasound

2. ABSTRACT

Background: For patients with a breast cancer tumour that are positive for node metastases, the ALND is the recommended and standard therapeutic axillar procedure. In the most aggressive tumour types, neoadjuvant therapy has showed high rates of pCR, appearing both in the primary tumour and in the axillar node metastases. Many trials have studied the possibility to avoid an unnecessary ALND if the axillar status has turned into a negative status after the NACT. The studies propose performing a SLNB after the NACT. However, the FNR associated to this SLNB are considered too high to be an accepted procedure. Ways to increase the SLNB accuracy should be tested. Placing a clip in the node could decrease the current FNRs.

Objective: The aim of this study is to determine if pathologic changes in clipped nodes predict better the nodal status by lowering the FNR, compared to SLNB alone without clips. We will also evaluate the sensitivity and specificity of the procedure.

Design: A randomized, single-blinded, controlled trial will be performed in the Hospital *Universitari Josep Trueta* of Girona within the Mammary Pathology Unit, from January 2018 to April 2021.

Methods: 110 patients with node positive early breast cancer will join the trial following a consecutive recruitment. Patients will be randomly placed in one of the two groups; Clip node and No clip node. For the statistical analysis of all data, T-student test, U-Mann Whitney test and Chi-Square test will be used. A confidence interval of 95% will be assumed and a $p<0.05$ will be considered to be statistically significant.

Key words: Neoadjuvant chemotherapy, Sentinel Lymph node biopsy, False Negative Rate, Pathological Complete Response, Node metastases, Clip.

3.INTRODUCTION

3.1 BREAST CANCER

3.1.1 IN NUMBERS

Worldwide, more than **one million new cases** of female breast cancer are diagnosed every year. It is the most commonly occurring neoplasia in women, accounting for over one-fifth of the estimated annual 4.7 million cancer diagnoses in females, and the second most common tumour, after lung cancer, in both sexes. It is also the **most common female cancer** in both developing and developed countries, with most (55%) occurring in the latter regions (1,2). More than **410,111 patients die** from **breast cancer every year** and the burden of this disease measured by incidence, mortality and economic costs is substantial and on the increase. Lifetime probability of acquiring breast cancer is 1 in 8 (2,3).

With the improvements in breast cancer screening programs and increased public awareness, more women now present with small primary breast cancers with **lower incidence** of **axillary metastases**. Therefore, there has been an evolution in the surgical management of early breast cancer, with standard treatment progressing from radical surgical resection to breast-sparing approach (2,3).

3.1.2 TUMOUR TYPES

Breast cancer is not a single malignant cell type, it has different types. Breast cancer is a **heterogeneous** disease with considerable **variation** in **histologic** and **biological** features. The genomic expression profile can be identified by molecular or inmunohistochemical techniques and allows classification of the breast cancer into subtypes. This improved understanding of patient-to-patient variability at the genomic and inmunohistochemical subtypes in breast cancer is improving our ability to direct the appropriate treatment to the appropriate patient. **Molecular subtypes** are recognized based on their gene expression profiles, but in clinical procedure, this subtypes can be identified by routine inmunohistochemical techniques. There are four basic molecular subtypes in

breast cancer. **Luminal A**, **Luminal B**, **HER2+** and **Triple Negative Breast Cancer** (TNBC).

Luminal A and **Luminal B** are the so-called hormone receptor positive tumours as they express progesterone and androgen receptors on its cell membranes. They are classified in A type and B type according to the rate of proliferation measured by Ki-67. They are known to be less aggressive tumour types compared to the HER2+ and TN tumours (2).

The **HER2+** gene encodes a tyrosine kinase receptor that mediates critical signalling functions in normal and malignant breast epithelial cells. An acquired alteration consisting of amplification and overexpression of the gene product occurs in approximately **20** to **25%** of human breast cancers. HER2+ overexpression is associated with an **aggressive clinical phenotype** that includes high grade tumours, increased growth rates, early systemic metastases and decreased rates of DFS and OS rates

TNBC describes a cancer that contains a BRCA mutation and immunohistologic pattern of both ER and PR hormone receptors negative and HER2 negative. It is found in **15** to **20%** of all breast cancers and it is usually the invasive ductal carcinoma subtype. TNBC has multiple subtypes that are classified by their immunohistologic pattern and unique identified gene mutation. The 80% of TNBC are classified as basal-like subtype. It contains similar immunohistologic characteristics of the cancer cell membranes and receptors that are similar to epidermal basal epithelial cells. A minority subtype of TNBC, termed claudin-low, has recently been identified in the non-basal TNBC subtype. Claudin-low TNBC cells are uniquely characterised by low to absent expression of epithelial cell-cell adhesion proteins, differentiated luminal cell-surface markers, and enrichment of epithelial to mesenchymal transition markers, immune response genes and cancer stem cell-like features. As neither of the hormone receptors nor the HER2 are expressed in TNBC, the hormonal and molecular therapies used in other subtypes are not effective. These cancers are characterised by greater **aggressive behaviour**, including early relapses, distinct pattern of metastases and higher rates of mortality. Those that have the claudin-low

subtype have an even poorer prognosis with less of a response to chemotherapy than other basal-like breast cancer (2,4).

3.2 SENTINEL LYMPH NODE BIOPSY

3.2.1 CURRENT RELEVANCE

It has been pointed out that the molecular profile in breast cancer will serve as a prognostic factor, as well as directing specific treatment for each individual case. But in early breast cancer, the **presence** of **axillary lymph nodes metastases** is one of the **most important predictors of overall survival** and recurrence. Therefore, the histological status of lymph nodes is nowadays regarded as the **most powerful predictor of survival** in patients with breast cancer and the standard of care for initial evaluation. So an accurate assessment of the status of the axillary lymph nodes provides either staging information or guidance regarding treatment options (3,5–11).
Traditionally, lymph node status was established by Axillar lymph node dissection (ALND), but this approach changed with the development of Sentinel lymph node biopsy (SLNB). ALND may cause side effects such as lymphedema (LE) wound infections, seroma formation and impaired range of shoulder motion, all having a negative impact in patient's quality of life. To avoid unnecessary complications of ALND, SLNB has provided an alternative to ALND in detection of lymph node metastases. Because of its **accuracy** and **limited morbidity** and **invasiveness** SLNB has become the **standard of care** for **initial evaluation** of metastasic spread to the axillary lymph node chain (3,5–12).Thanks to SLNB the surgical management of primary breast cancer has become less aggressive, with the possibility to use a breast-conserving surgery without compromising local control or long-term survival (5,10,11).
Patients with breast cancer now present with smaller tumours than in the past. The incidence of axillary nodal involvement is, therefore, also lower than before. Lymph node metastases were found only in 40% of patients who underwent ALND (9). Thus, the remaining patients did not take any benefit from the procedure, but incurred the risk of LE. So, it is difficult to justify exposing patients with breast cancer to the risk of axillary dissection, which carries

significant morbidity, just to satisfy rigid criteria for staging, especially if a large proportion of patients have no axillary involvement (5,9,11).

3.2.2 DEFINITION AND HISTORY OF ITS DEVELOPMENT

The sentinel lymph node (SLN) is defined on the basis of the hypothesis that lymph flow is orderly and predictable, and that the tumour cells disseminate sequentially. SLN has been defined as the **first lymph node that receives direct lymphatic drainage from the anatomic region of the primary tumour** and is immunologically responsible for that region. The definition of its concept implies that is the first lymph node to become involved when the tumour cells invade the lymphatic chain. From a surgical point of view, a SLN are the nodes that show a representative activity and are located in the area previously selected by a gamma probe (3,5,6,8,10,11,13). The concept of a mapping lymphatic drainage began in the 1950's. By the 1970's it was noticed that some nodes received drainage before others and so were termed "sentinel lymph nodes". Lymphatic mapping with the purpose of discovering the sentinel node was first described in 1992 for cutaneous melanoma. In **1993** it began to be applied in the field of **breast cancer**, by injecting the radiotracer and the use of gamma probe. Since those pioneering reports, SLNB has become the standard method for axillary lymph node staging (6,14).

3.2.3 INCLUSION AND AND EXCLUSION CRITERIA

As the SLNB is a diagnostic invasive procedure, and there exists risks of side effects and further complications, its use should be always well justified. As any other invasive procedure, it has well-defined inclusion as well as exclusion criteria to limit its use as an interventional diagnostic procedure (5–7,11,13).

- Methods to assess the indication of patients to a SLNB procedure

Ultrasound (US) is the **most adequate technique** when assessing the state of the axillary nodes to select the patients that would be candidates to perform a SLNB. Its main utility comes from the possibility to detect axillary **nodes** that **show morphologic changes**. Such changes would be indicative of node invasion and could not be detected by physical exploration methods. It also allows to perform an **US guided needle biopsy** of the altered nodes detected

in order to confirm the diagnosis of node invasion before performing the SLNB. Every node that shows possible morphologic signs that could translate tumour invasion should be confirmed to be malignant with an Aspiration needle puncture (ANP), as the morphologic study only with US would carry too false positive results. When **both techniques are used** the **specificity** almost reaches **100 %,** while complications of both procedures are very low. There is not enough data to recommend one of the two possible needle biopsy techniques. The decision should be based on the experience and personal preferences of the physicians that will perform the procedure.

So its relevancy of both techniques in such scenarios is that it would **avoid** the **performance** of the **SLNB**, if the nodes detected by US and histologically studied afterwards, turn to be metastasic. It should be highlighted that the greatest the tumour size is, the greatest its cost-effectiveness will be. US also allows to assess the approximately number of nodes affected, which will be important in the tumour staging, as well as its extra capsular and massive axillar possible invasions.

A **MRI** before surgery is also **recommended**, as it can be useful to identify less accessible nodes by US (level 2 and 3, internal mammary chain and Rotter nodes). It is also useful to assess the exact number of nodes affected and to compare with the contralateral axilla (5–8,11,13).

- Inclusion criteria

Table 1. Inclusion criteria for SLNB. Adapted from (13)

-Infiltrating carcinomas T1, T2 and T3 stage, as long as the axilla is negative tested by US and by pathological methods	
-Intra ductal carcinoma with	
	Mastectomy indicated
	High risk of micro invasion according to the following criteria: -Diameter => 3cm -High histological grade -Palpable -Comedonecrosis

- Exclusion or non-indicative criteria

Table 2. Exclusion criteria for SLNB. Adapted from (13)

-Preoperative **proof of positive nodal invasion** tested by image techniques (US) and at least a positive citology of the nodes that are suspected to be metastasic by US*
-**Inflammatory carcinoma**
-**Locally advanced carcinoma** with **nodal invasion**, without any previous systemic treatment

*The main reason would be that if there exists an involvement of the axillary nodes, leads to a mechanical obstruction of the lymphatic sinuses, which in turn leads to directional flow changes and transport of the radiocolloid and/or the blue dye to a non-sentinel node. This will imply that the result obtained would be with great chances a false negative (5,11).

- Non-exclusion criteria

Table 3. Non-exclusion criteria for SLNB. Adapted from (13)

-Previous excisional biopsy, as long as there are any excluding criteria simultaneously
-Previous breast plastic surgery
-Multi centric and multifocal tumours
-Pregnant or breastfeeding women (always suspend the breastfeeding in the previous 24 hours before the procedure).*

*In such cases, it is recommended to use the minimal possible dose of radiocolloid and inject it the same day of the SLNB procedure. In such cases, it is strictly contraindicated the use of any type of dye.

- Special considerations to recommend SLNB

Table 4. Special considerations for SLNB. Adapted from (13)

-Patients that initially presented with **cN1/N2**, that is axillary **node invasion,** but after Neoadjuvant Chemotherapy (NACT) present with clinically and US negative axillary node invasion, having high chances that the status of the axilla has turned into negative for axillar node invasion (cN0), in order to avoid ALND.

3.2.4 SIDE EFFECTS

Lymphedema

Historically lymphedema has been the **most feared complication** of the surgical treatment of the breast cancer. With the introduction of SLNB for axillary staging, it has been largely assumed that SLNB would be associated with minimal morbidity as compared with fully ALND. Several studies have suggested that **SLNB** does indeed **reduce,** but **does not eliminate LE,** as the SLNB procedure also disrupts the lymphatic system (table 5). LE implies a series of **functional** and **psychological morbidities** to the patient that suffers it, such as difficulty with work and life activities as well as an altered body image (3,9,15–18).

- Physiopatological formation process

LE is a result of a **functional overload** of the **lymphatic system** in which lymph volume exceeds transport capabilities. This occurs after surgical disruptions of lymphatic channels when undergoing procedures such as SLNB and ALND. There is an **increase in oncotic pressure** in the tissue due to the accumulation of the interstitial macro molecules, which leads to more swelling (9).

- Clinical definitions

By definition **acute LE** occurs and resolves within the **year following surgery**, representing a successful adaptation to an altered anatomy due to surgical axillar procedures. Lymphatic channels may reform or alternative routes may be established to permit again a lymphatic return.

Chronic LE can occur at **any time after treatment** and it is **irreversible**. Acute LE that remains persistent progresses into chronic LE. Left untreated, chronic LE is a progressive disorder characterized by **chronic inflammation**, **swelling**, **fibrosis** and **increased risk of cellulitis**.

The time between surgery and the development of LE is quite unpredictable, as it can happen years after. In most of the cases (77%) the LE occurs within **3 year**s, and the other 25% remaining, appears after 3 years. Women without lymphedema after 3 years have an ongoing risk of developing LE of

approximately 5% at least for 20 years. The LE after ALND follows the same pattern. It must be highlighted that the current published literature regarding LE after SLNB reports only short-term outcomes (less than 3 years) (15,16).

It is difficult to connect both physically changes assessed by an objective way of measurement and sensations assessed by patient's report (9,16). As it could exist discordance between objective and subjective measurements of LE, **one method** is **not adequate** to accurately **define** the **presence of LE** in patients after SLNB or ALND surgical procedures. When assessing the presence of LE there should be used standardized objective measurements together with patient interview data (16).

Table 5: Lymphedema measurement methods. Adapted from (15)

Study	Measurement Method	Definition of Lymphedema	Lymphedema Less after SLNB
Burak, 2002	Circumference	Ratio with/difference from contralateral as continuous variable	S
Haid, 2002	Circumference	>10% difference from contralateral	S
Golshan, 2003	Circumference	>3cm difference from contralateral	S
Veronesi, 2003	Circumference	>2cm difference from contralateral	S
Leidenius, 2005	Circumference	=>2cm difference from contralateral	S
Wilke, 2006	Circumference	L > 2cm	-
Langer, 2007	Circumference	=>2cm difference from baseline or contralateral or edema	S
Lucci, 2007	Circumference	L => 2cm	S

S= Significant

- Risk factors

Table 6. Risk factors for arm-swelling. Adapted from (16) (19).

Risk factors associated with LE development
Obesity
Increased BMI
Weight gain
Radiotherapy
Older age
Low physical activity*
Injury of the arm*

*The influence of this factors has not been yet well examined or established the type of relation.

Table 7. Adapted from (20)

Risk factors associated with a greater severity of LE
High number of lymph nodes removes
Greater lymph node dissection procedures

Increasing physical activity and **weight loss** have been the focus of interventions designed to **prevent**, **stabilize** or **reduce LE severity** (20,21)

- Successfully reducing Lymphedema

SLNB is associated with **lower rates** of **LE** compared to ALND, almost to negligible rates (3,5–7,9,11,14–16).

Several prospective studies have concluded that the rates of LE vary from 0 to 16,8% at 6 and 36 months after SLNB (15).

Studies have also concluded that there was also a **significant difference** in **severity** of **LE**, being **less** after **SLNB** compared to ALND (table 8) (9).

Table 8. Characteristics of LE severity by axillary surgery. Adapted from(9)

	SLNB	ALND	P value
Severity (%)			0,03*
Level I	65	41	
Level II	35	59	

*p<0,05

Sensitive side effects

After breast cancer surgery patients often describe a variety of sensations in and around the axilla, breast, or chest wall, on the affected side. At times, these sensations can be severe and distressing. These sensations generally result from **injury** to **or resection** of **specific nerves** in the operative field. These sensations are long-term felt by the patients, being suffered after **both ALND** and **SLNB procedures**. Most of them occur during the three months after surgery, and even observed in both procedures, are generally **not severe** or distressing (17,18).

The lateral branch of the second intercostal nerve, known as the **intercostal brachial nerve,** is responsible for many sensory disturbances after breast cancer surgery field. This sensory nerve, which supplies the axilla and medial upper arm, is frequently sacrificed to gain wide access to the axillary contents. Even carefully preserved, however, the nerve may be stretched or injured. Any of these events could result in postoperative sensory morbidity (17,18).

Disruption of the axillary nerves and lymphatic nodes is believed to occur **less often** with **SLNB** than with ALND. Therefore, postoperative sensations after SLNB are also believed to be less extensible and **less severe** compared with these after ALND (17,18).

The **most prevalent** sensation both in ALND and SLNB is **tenderness**. Surprisingly, 33% of SLNB patients and 40% of ALND patients still experienced this sensation after 5 years. Other sensations that remained notably prevalent 5 years after SLNB included twinges (34%), soreness (27%), aching (27%) and tightness (27%). The two most prevalent sensations are more prevalent after ALND (17,18).

Patients who undergo **ALND** experience **far more** tightness, stiffness, numbness, tenderness and soreness (figure 1) than those who underwent SLNB. Five years later, numbness and tightness remain **far more prevalent**

after ALND compared with SLNB. **Numbness** is one of the **most severe** and **distressing** sensations (17,18).

There is not only a clear difference between its prevalence among the two procedures, there is also a **difference** regarding the **level of distress** and the **severity**. For example, numbness, stiffness, tenderness, soreness and tightness are **far more severe** after **ALND** (Figure 2) compared to SLNB, while pain, tightness and burning are far more distressing among the patients who undergo ALND compared to SLNB.

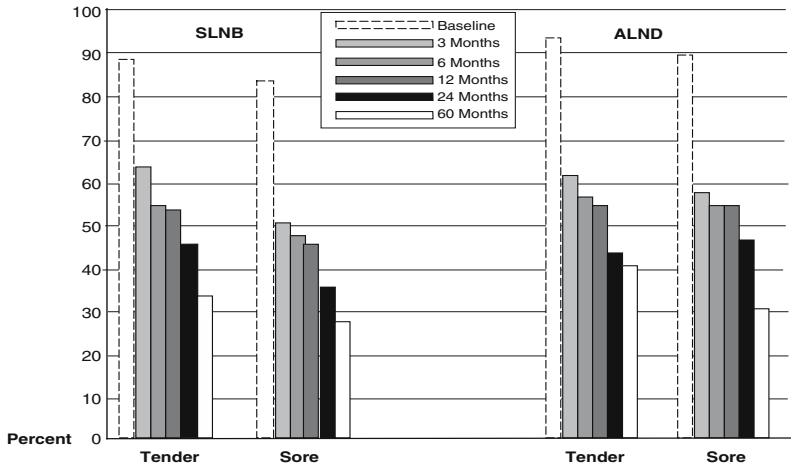

Figure 1. Prevalence at baseline and at 3, 6, 12 ,24, and 60 months of the two most reported baseline sensations for patients who underwent SLNB and ALND (17)

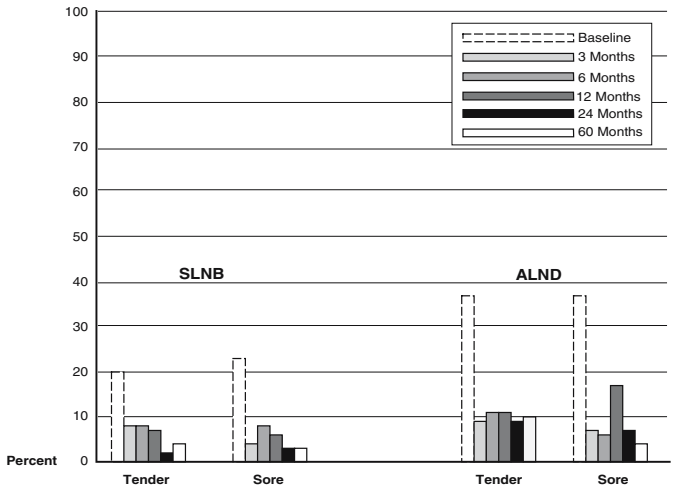

Figure 2. Severity at baseline and at 3, 6, 12, 24, and 60 months of the two most reported baseline sensations for patients who underwent SLNB and ALND (17)

In conclusion, **SLNB** seems to be associated with **less sensory morbidity** of the affected arm (7,17,18).

Pain and impaired mobility

Although LE is the most common and feared complication of surgery procedures involving the axilla, both ALND and SLNB can cause postoperative arm pain and motor restriction (18).

Moderate to severe pain is reported between 20% and 32% after ALND and it is not significantly related to time since surgery. Data shows **less postoperative arm pain** after **SLNB** (figure 3). SLNB is associated with mild pain, significantly less than ALND, and **improves** during the months following the surgery. So, **SLNB** shows a **significant difference** in **pain severity** compared to ALND (18).

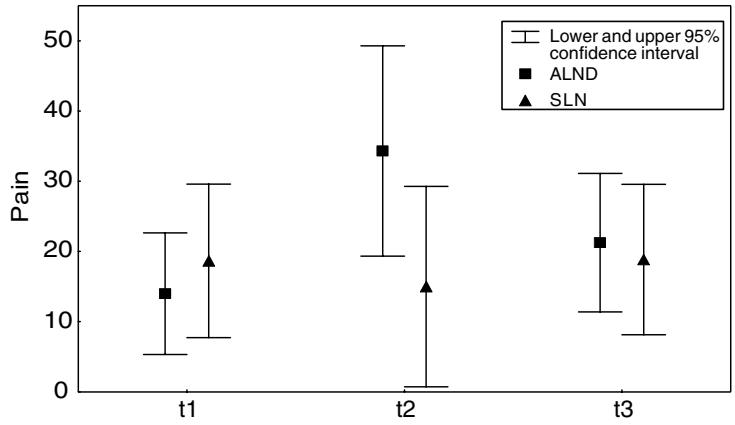

Figure 3. Comparison of pain (means) after ALND and SLNB over time show significant higher levels of pain at t2 (after discharge) in patients who underwent an ALND procedure.(17)

SLNB is also associated with **lower rates of arm mobility**. Arm or shoulder abduction, flexion and horizontal adduction show significant impairment after ALND when compared with the preoperative range of motion (table 9).

Table 9. Means arm/shoulder motion by goniometric measurement. Adapted from (18)

9-12 months after surgery	ALND	SLNB	P-value
Abduction	143.8 (22,8)	159.9 (13,9)	**0,007***
Flexion	146.0 (15.9)	154.6 (15.0)	**0,03***
Extension	47.1 (11.2)	52.2(27.1)	0,39
Horizontal abduction	101.1 (15.9)	106.5(21.3)	0,76
Horizontal adduction	34.5 (14.1)	35.6(19.1)	**0.011***

*p<0,05.

As well as LE, pain and impaired arm and shoulder mobility after ALND and SLNB, are associated with a **negative effect** on the **overall quality of life** of breast cancer patients (18).

3.2.5 ADVANTAGES

It has been mentioned the possible side effects of a procedure like SLNB, in which there is a disruption of the lymph circuit and it might be a disruption of some other structures such as nerves. It has been exposed that SLNB as well as ALND can cause postoperative side effects such as LE, sensory morbidity, reduced arm/shoulder mobility and pain. Although these would never be considered as advantages, the **lower morbidity rate** compared to ALND would indeed be considered as an **important advantage** of the procedure. The most important and long-term reported side effect, LE, has widely been reported to be lower and less severe with SLNB. It is an important and remarkable advantage as it has been underlined its **relevancy** in the **postoperative quality of life** of the patients. The other side effects had been reported to show in lower rates in SLNB procedures.

The lower rates of morbidity linked to the practice of SLNB would also imply a **shorter time to resumption of normal daily activities** as well as improved quality of life and arm functioning scores (6).

Another advantage would be that being less aggressive and carrying less arm and shoulder morbidity, it has **no difference** in **OS, DFS and RC.** They are statistically equivalent between the SLNB and ALND. These rates mean that

there is **no difference** in **axillary recurrence** between **SLNB** and **ALND**. The same happens with the mortality rates; there are no significant differences between the two procedures (3,5–7,11,14).

The **accuracy**, **sensitivity** and **success rates** of **SLNB** are **very high**, close to 100% (5,8,11) (table 10).

It will also allow for **selection of patients** before invasive axillary or internal mammary surgery as well as optimising the surgical approach (5,11).

Table 10. Adapted from (8)

Study	Study type	SLNB success (%)
Yamamoto et al, 2013	Cohort (prospective)	99.6%
Sugie et al, 2013	Cohort (prospective)	99%
Schaafmsa et al, 2013	Cohort (prospective)	100% (radiocolloid) 88% (blue dye)
Van der Vorst et al, 2012	Cohort (retrospective)	100% (radiocolloid) 84% (blue dye)
Hirche et al, 2012	Cohort (prospective)	97.9%
Wishart et al, 2012	Cohort (prospective)	99% (blue dye) 91.3 % (radiocolloid)
Tagaya et al, 2011	Cohort (prospective)	100%
Aoyama et al, 2011	Cohort (prospective)	100%
Hojo et al, 2010	Cohort Control (prospective)	100% (radiocolloid) 92.9% (blue dye)
Murawa et al, 2009	Cohort Control (prospective)	97%
Kitai et al, 2005	Cohort (prospective)	94%

Overall, SLNB is a **safe**, **reliable** and **reproducible** operation that provides an **accurate assessment** of **nodal metastases** for the breast cancer patient. It continues to add to the trend in breast surgery in which the surgeon provides **less surgery** with a minimal invasive technique of axillary staging. It also **avoids** the **unnecessary complications of the ALND**, while maintaining a standard of care that minimizes breast cancer morbidity and mortality (3,6–9).

3.2.6 DISADVANTAGES

The critical issue in SLNB is the **false negative detection rate** as this can lead to **incorrect treatment decisions** in patients with early breast cancer who will mostly benefit from adjuvant therapy in the presence of axillary disease. The frequently accepted false negative rate is between **5** and **7,6%** (table 11) (11).

Table 11. Meta-analysis of the SLNB studies in breast carcinoma (11)

Study	Year	Accuracy (%)	FNR (%)
McMasters	1998	98	6.2
Mittenburg	1999	98	5.1
Martins	2000	98	5.8
Gemignani	2001	97	7.6

3.3 NEOADJUVANT CHEMOTHERAPY

3.3.1 DEFINITION

Neoadjuvant Chemotherapy (NACT) is defined as the **administration** of systemic **therapy prior** to **surgical removal** of a breast tumour. It was originally designed to be used in patients with locally advanced disease in order to convert inoperable tumours into operable tumours (22–24). Its relevancy as preoperative treatments is supported by the **high rates of tumour downsizing** (94%) and the **moderate rates of pCR** (20-40%) (12,24–28) (table 12).

Table 12. pCR rate after NACT in hormone receptor negative tumours (Her2 and TN) compared to hormone receptor tumours (luminal A and B). Adapted from (26).

Studies	pCR (%) for HR+	pCR (%) for HR-
MD Anderson	61,50%	70%
NOAH	18%	38%
NeoALTTO	22,70%	36,50%
NeoSphere	20%	36,89%
NSABO B-41	46,70%	65,50%

HR=Hormone receptor

3.3.2 USE AND OBJECTIVES

- **Downstage the tumour**

NACT has become a **standard treatment** for **locally advanced breast cancer** to downstage tumours which aids in surgical resection (22,23,26). Additionally, downsizing the primary tumour can make breast-conservative surgery possible for some patients who were initial candidates for mastectomy and therefore improves cosmetic outcome. NACT **avoids mastectomy** in 25% of the patients (22,25,26).
So the main purpose of the NACT is to reduce the size of the primary tumour, eventually **allowing radical surgical procedures** (4,27).

- **Role as a prediction factor for prognosis**

The assessment of tumour behaviour in situ during NACT and its correlation with clinical outcomes is an **excellent model** to determine the **predictive role** of **tumour characteristics.**
Response to NACT is evaluated by the change in tumour size from pre-treatment clinical and/or radiologic measurement to post-treatment status. The **spectrum of response** to **NACT** varies from complete response, partial response to non-response. In line with accepted definition, **pCR** denotes a condition when **residual tumour** is **neither** detected in **breast** or **axilla**. Patients who achieve **complete response** to NACT experience **better outcomes**, that is, long-term disease-free survival and better OS when compared to those patients whose tumours do not respond to therapy. Many studies suggested that there is a **relationship** between **pCR** and the **patient outcome**. Patients who showed pCR, defined as ypT0 ypN0 or ypT0/is ypN0 experienced better survival (4,12,22,23,25,26). The primary systemic treatment is also useful when choosing the afterwards adjuvant treatment, as its response allows the study of different prediction factors (25).

- **Reduce nodal status**

The **pCR occurs** not only in the primary tumour but **als**o in the involved **axillary nodes,** as the previously mentioned definition of pCR, it also includes changes in the status of the axilla. Therefore, **ALND might be avoided** or replaced by a more conservative procedure in a certain subtype of node positive breast cancer patients **after NACT** (12,26).

The ways of measurement of NACT response are the same for nodal status as the ones used for tumour size (22). Patients with residual tumour in lymph nodes have worse prognosis compared to patients who have no residual tumour in lymph nodes.

3.3.3 EVALUATION OF RESPONSE TO NACT

Response to NACT can be assessed by **clinical examination**, **breast imaging** studies and ultimately, by **pathologic examination** of post treatment specimen. **Clinical examination** is performed by assessment of the size of the tumour by palpation. The task is more difficult in tumours that have responded to therapy, as it is challenging to palpate the real tumour versus the treatment-induced changes. Breast imaging modalities such as mammography and ultrasound are not considered adequate for quantitative assessment of change in tumour size. **MRI and PET-CT**, however, have been shown to provide a better assessment of the tumour response to NACT and better predict response to therapy (22,25). The **gold standard** to determine if there has been reach a pCR is through **pathology examination**. The definition of pCR includes the term ypT0N0, which can only be determined by a pathologic examination. As to get a sample of the tumour would necessarily mean to do another biopsy, in such tumours that have had a NACT regime before surgery, the sample of the tumour will be obtained in the same surgery procedure. So, this would mean that the definitive establishment of the tumour response would come after surgery, with the pathology examination (22).

Currently, the pathological response to NACT can be determined by changes in tumour size, changes in ki-67 proliferation rate, the status of hormone receptors if the tumour turns to be Her2+ subtype, size of tumour bed and tumour cellularity. The reduction in tumour cellularity is assessed by the **Miller and Payne score** (table 13). It is a histopathology **scoring system** to assess the

pathological response. It compares the cancer cellularity of the core biopsy (before treatment) with the resected tumour (after treatment). It also estimates the probability of DFS in the different grades of pathologic response. The Miller-Payne scoring system can be used in both breast tumour and node invasion (29–31).

Table 13. Miller-Payne grading system. Adapted from (30)

Grade 1: No change or some alteration to individual malignant cells, but no reduction in overall cellularity
Grade 2: A minor loss of tumour cells, but overall cellularity still high**; ≤ 30%** loss
Grade 3: Between **30%-90%** reduction in tumour cells
Grade 4: A marked disappearance of tumour cells such that only small clusters or widely dispersed individual cells remain, **> 90%** loss of tumour cells (almost pCR)
Grade 5: No malignant cells identifiable in sections from the site of the tumour; only vascular fibroelastic stroma remains, often containing macrophages **(pCR**)

However, this assessment may be complicated by the presence of associated chemotherapy induced tissue reaction resulting in underestimation of cellularity. This is why it is crucial an accurate assessment of tumour cellularity before the NACT.

3.3.4 TUMOUR SUBTYPES

The selection for NACT and its impact on breast cancer outcome, mainly depend on molecular subtypes and the degree of proliferation rate. **High** tumour **grade**, **high proliferation** rate, tumour **necrosis** and **presence** of tumour **associated lymphocytes** are considered **predictors** for a **better response**. Therefore, the impact of NACT in reaching pCR is more conspicuous in aggressive tumour subtypes such as **TN** and **HER2+.** As in Luminal A and Luminal B tumours the pCR is much less significant, the NACT recommendation criteria for these two molecular subtypes are much more restricted (table 12) (4,22,25,26).

Table 14. NACT recommendations (25):

Tumours that overexpress or show high rates of amplification of **HER2+**, from **T2** stages or nodal invasion (regardless of the T stage)
Triple Negative tumours from **T2** stage or nodal invasion (regardless of the T stage) excepting the cases of methaplasic carcinoma due to the low chances this tumour have to respond to an NACT treatment. Neither is recommended in the adenoid carcinoma, due to its good prognosis.
Luminal tumours HER2 negative and Ki-67 >25%: -If < 71 years from T2 stage or nodal invasion -If >70 years or severe comorbidities

3.3.5. REGIMES OF NACT

Table 15. Adapted from(25)

NACT regime for Triple Negative Breast Cancer
First choice: Nab-paclitaxel 125mg/m^2 **x** 12 cycles followed by Epirubicina-Ciclofosfamida **x** 4 cycles
Alternative: Nab-paclitaxel 125mg/m^2 **x** 12 cycles followed by carboplatin 1,8 every 21 days **x** 12 weeks

Table 16. Adapted from (25)

NACT regime for HER2+ Breast Cancer
Adriamicina-Ciclofosfamida 21 days **x** 4 cycles followed by pertuzumab **+** trastuzumab **+** paclitaxel 80 **x** 12 cycles**.**

Table 17. Adapted from (25)

NACT regime for Luminal tumours
Adriamicina-Ciclofosfamida + Docetaxel.

3.4. SLNB AFTER NACT

For many years, the standard treatment of the axilla after NACT has been ALND in node-positive patients. There has been a lot of debate on whether is better doing the SLNB after NACT. The main reason to claim its implementation would be to **take advantage** of the **increasing pCR obtained with the newest targeted therapies**. This, would be translated into more conservative therapies in the axilla if the pCR is also obtained in the axilla, meaning that the status of the axilla would have changed to a positive status to a negative status. It would allow the **patient** with **clinically occult axilla** to **avoid ALND** if the **nodal metastases** are **eradicated** with **NACT**. Moreover, the results of the nodal status after NACT would also have a prognostic relevance as it would work as an important indicator of the overall tumour prognosis, as some have suggested that the nodal status after NACT reflects the prognosis more accurately than the initial axillary status (10,12,24,26,27,32).

Even that a SLNB after NACT would have important and relevant innovations, such as changing the current strategy of axillary dissection to a more conservative approach avoiding ALND, as well as obtaining further prognostic information with the after-NACT nodal status, there is **still debate** on **performing a SLNB after NACT** (10,26,33,34).

One of the important reasons would be that perhaps this **SLNB after NACT carries** too many **limitations** for its implementation. The most important limitations and more challenging ones are the **identification rate** and the **FNR**, both being very important measures of procedural accuracy. It is measured by comparing the pathological status of the SLN to the remainder of lymph nodes in the axilla following ALND. False negative results occur when the SLN does not contain cancer, but cancer cells are then found in the remaining axillary nodes (28). The FNR can be high because alternative pathways and non-

proportional regression of lymph node metastases (27). Also, the uneven **effect of NACT** could **increase the FNR** by inducing complete pathological response on a previously positive SLN while not eliminating the tumour from other lymph nodes (35). Also, the **identification rate** of the second SLN can be **hindered** by **obstructed lymph vessels** or channels and can be impaired due to post-NACT fibrosis, potentially diverting the lymphatic flow to non-sentinel nodes (11,27,34,35).

There had been **two very important studies** studying the FNR of the SLNB after NACT (Table 18). **ACOSOG Z0171** enrolled almost 700 patients who went ALND and SLNB after NACT. The primary endpoint of the study was the FNR for clinically node positive patients who have at least 2 SLNs excised should be ≤ 10%. The **SLN identification rate** was **92,5%** and the **FNR** of **12,6%.** So the study did not reach the FNR the authors had established in advanced (6,7,10,12,26–28,34,35).

The second study was the **SENTINA** study. They also studied the use of SLNB in different timing in the neoadjuvant setting. It showed similar results to the reported data from ACOSOG Z0171. Due to the **high FNR** of the SLNB after NACT, **authors** do **not recommend** the **use** of a **SLNB** in the neoadjuvant setting.

Some **more studies** (table 19) have concluded **the same as the ACOSOG Z0171** and **SENTINA** and it is that the **current FNR** of the second SLNB **are unacceptable** (7,23,27,33–37). For these reasons, **ALND** is the **current approach** for patients that showed a positive node status before NACT, regardless of the pCR rate of the tumour after NACT. A **FN result** could be **detrimental to the patient** because it results in inaccurate staging with important implications for adjuvant therapy and the possibility of persistent axillary nodal disease (33).

Although these studies do not recommend to do a SLNB after NACT, they open a way for HER2+ and TN tumours to be potential candidates for avoiding unnecessary axillary dissection due to its high axillary node negative status rate after NACT (10,26,27).

Table 18: Results from the prospective trials in SLN after NACT in clinically positive patients. Adapted from (12)

	ACOSOG Z0171	SENTINA
Patients (N)	756	592
FNR => 2 SLNs	12,60%	9,60%

Table 19. Adapted from (28)

Study	Design	SLN IR	FNR
Yagata et al, 2013	Prospective	85,30%	15,70%
Rebollo-Aquirre et al, 2012	Prospective	92%	8,30%
Kim et al, 2015	Prospective	96%	10%
Koslow et al, 2014	Prospective	98%	8,30%
Boughey et al, 2013	Prospective	92,90%	12,60%
Rebollo-Aguirre et al, 2013	Prospective	84,90%	8,30%
Alvarado et al, 2012	Prospective	93%	20,80%
Takei et al, 2013	Prospective	NR	8,20%
Thomas et al, 2011	Prospective	88,67%	20%
Carnavese et al, 2011	Prospective	93,80%	5,10%
Ozmen et al, 2010	Prospective	92%	13,70%
Shen et al, 2007	Prospective	92,80%	25%
Classe et al, 2009	Prospective	90%	11,50%
Boileau et al, 2015	Prospective	87,60%	8,40%
Park et al, 2013	Prospective	94,90%	22%
Brown et al, 2013	Prospective	NR	22%
Kang et al, 2011	Prospective	95,70%	17,10%
Lee et al, 2007	Prospective	77,60%	5,60%
Kuehn et al, 2013	Prospective	80,10%	14,20%

FNR= False negative rate, IR= Identification rate, NR= Not reported

- How to increase SLNB accuracy and future directions

Researchers have investigated other **ways to lower the FNR of the SLNB after NACT** in patients with initially clinically positive axillary nodes. Some studies have **placed a clip** in the positive axillary node at the time of the biopsy guided by ultrasound, so the both SLN and suspicious nodes are removed during the SLNB after NACT (12,28).

The relevance of residual disease after NACT, the need for ALND in those patients converted to node negative after NACT, the role of radiation therapy to

the nodal basins in the presence of axillary pCR are the great challenges at this time. With more patients undergoing NACT and with the improvements in pCR, tailoring decisions of loco regional treatments after NACT based on the pathologic response would be of extremely importance (12).

4. JUSTIFICATION

Breast cancer is the most commonly occurring neoplasia in women. More than 410,111 patients still die from breast cancer every year. The burden on this disease measured by incidence, mortality and economic costs is substantial and on the increase.

The presence of nodal metastases in breast cancer is an important prognostic factor that is used to guide loco-regional and systemic treatment decisions. Traditionally, the lymph node status was assessed performing an ALND, however, this procedure was associated with significant morbidity compared to SLNB. ALND may cause side effects that are irreversible and have a great impact in patient's quality of life. The most prevalent and most feared is lymphedema, although post-surgery pain and impaired range of shoulder motion can also appear after an ALND. Several studies have suggested that SLNB does indeed reduce, but does not eliminate these post-surgery complications (3,6,8,9,15–17).

With the improvements in breast cancer screening programs and increased public awareness, more women now present with small primary breast cancers with lower incidence of axillary metastases. So to avoid unnecessary complications of ALND, SLNB has provided an alternative to ALND in detection of lymph node metastases, as it is difficult to justify exposing patients with breast cancer to the risk of axillary dissection, which carries significant morbidity, just to satisfy rigid criteria for staging, especially if a large proportion of patients have no axillary involvement. Because of its accuracy and limited morbidity and invasiveness, SLNB has become the standard of care for initial evaluation of metastasic spread to the axillary lymph node chain. SLNB has represented a step forward in both the surgical management of early breast cancer and the overall patient's quality of life after surgery. As for the surgical management of early breast cancer, it has become less aggressive, with the possibility to use a breast-conserving surgery without compromising local control or long-term survival. As for the improvement in patient's quality of life it has implied a shorter time to resumption of normal daily activities.

Patients presenting with nodal metastases often receive Neoadjuvant Chemotherapy. NACT brings many advantages, not only in downstaging the tumour before surgery, but also in changing the axillar status to a possible pathologic node negative status, and ultimately, giving important prognostic information about the tumour depending on the pathologic response of the nodal status to the NACT. After NACT is done, ALND has been the standard surgical approach in patients who showed a positive node status before the systemic therapy.

With all this information on response rates to NACT and the radical success of lymphatic mapping with SLNB, many investigation groups embarked on testing SLNB after NACT in patients with initial biopsy-proven axillary node metastases. The most important reason to start considering SLNB after NACT was on whether the clearly morbidity associated to ALND is justified to such patients, carrying unclear benefit, if there has been reached a nodal pCR with NACT.

The initial retrospective and prospective studies demonstrated that a SLNB after NACT carried too many limitations. The most relevant ones were the too high rates of false negatives and the too low identification rates, both being very important measures of procedural accuracy (6,7,10,12,26–28,35,37). A FN result could be detrimental to the patient because it results in inaccurate staging with important implications for adjuvant therapy and the possibility of persistent axillary nodal disease.

False negative results occur when SLN does not contain cancer, but cancer cells are found in the remaining axillary nodes. Both identification rate and false negative rate are higher than the SLNB before NACT because there may be changes in lymph vessels such as creation of alternative pathways due to NACT (7,10). The placement of a clip at the time of the ANP at the positive axillary node would actually make sense, because the lymph node most important to assess after NACT would be the actual lymph node that had metastastic carcinoma prior to therapy, and so it would lower the FNR (12,38,39).

With more patients undergoing NACT and the improvements in pCR, it is crucial to improve the loco regional management of the axilla after NACT based on the pathologic response, and so there is of extreme importance to find a way to lower the FNR of the second SLNB so it turns into an accepted axillar management to many patients, avoiding the unnecessary morbidity of an ALND.

5.HYPOTHESIS

The placement of clips in nodes with citology-confirmed metastases before initiating NACT would lower the FNR of a SLNB after NACT and so it would improve the nodal staging evaluation compared to a SLNB alone without clips.

6.OBJECTIVES

6.1 PRIMARY OBJECTIVE

- The aim of this study is to determine if pathologic changes in **clipped nodes** predict **better** the **nodal status** by **lowering the FNR**, compared to SLNB alone without clips.

6.2 SECONDARY OBJECTIVES

- Determine the **percentage of patients** that reach a pathologic node negative status (**pCR)**.
- Define the **sensitivity** and **specificity** of the SLNB with evaluation of clipped nodes.
- The **percentage of patients** where the **clipped node** was **not identified** as an **SLN**.

7 METHODS

7.1 STUDY DESIGN

The most accurate design that would allow us to confirm or refuse our hypothesis with a high level of evidence we would propose a **prospective randomized**, single-blinded, consecutive, comparative and control interventional **clinical trial**. The control group will be the patients that will not be placed a clip in the pathological node and the experimental group will be the patients that will be placed a clip in the pathological node (Figure 4).
It will be a **single-blinded** trial because both the surgeon and radiologist that perform the interventions will know if they are placing a clip or not. The patient, however, will not know whether she is being placed a clip or not, as well as the pathologist.

7.2 SETTING AND POPULATION OF STUDY

This clinical trial will take place at a single comprehensive cancer centre at the Hospital *Universitari Josep Trueta de Girona,* where the patients will be selected, operated and followed.
The study population will be patients with **early breast cancer** and **citology-proven axillary node metastases** at the time of diagnosis with a **clip placed** in the metastasic node, being **eligible for NACT** prior to surgery and that have potentially reached a **pathological negative node status after NACT**. The patients will need to fulfil the specific criteria for undergoing NACT and SLNB separately. Patients not fulfilling the specific criteria for these two mentioned procedures will not be considered as part of the study population.

- Patient inclusion criteria

In order to enter and take part in our clinical trial, patients will necessarily need to meet all the following criteria:

Table 20. Patient inclusion criteria.

1.To have an image and histological confirmed **diagnosis of early breast cancer** (stages I-III) of any molecular subtypes (and later fulfil the specific criteria to recommend NACT for every tumour subtype)
2.**Female** gender
3.**Absence** of any **medical condition** that makes the surgery too risky
4.Reading and **signing** the 3 **informed consents** (ANNEX 2,3 and 4)
5.Fulfiling the **special considerations** criteria for **SLNB** (table 4)
6.Citology- proven **node metastases** by ANP (**cN1** nodal status)
7.**Elegibility for NACT** tested by a radiological extension disease evaluation MRI and a bone scan (a stage corresponding with absence of any distant metastases)
8.To **complete** a NACT regime
9.To have a radiological **evaluation** of the **response** to **NACT**

- Patient exclusion criteria

Patients that meet at least one of these following criteria will be excluded from our clinical trial:

Table 21. Patient exclusion criteria

1.**Male** gender
2. Patients that will **not** undergo **surgical treatment**
3. **Refuse** reading and/or **signing** the 3 **informed consents** (ANNEX 2,3 and 4)
4.Absence of citology-proven **node metastases** or a non-registered nodal status
5. Patients with **no histological confirmation** of a breast cancer diagnosis
6.**Not elegible for NACT** after a radiological extension of disease evaluation with MRI and a bone scan (a stage corresponding with distant metastases)
7. **Not** being able to **complete** a **NACT regime**
8.Presence of any **medical condition** that would make the **surgery too risky**
9. Cases with **no registered** data of the **NACT response**, tested by radiological methods.

7.3 SAMPLING

Sample selection

A consecutive non-probabilistic sampling will be done in this study. Patients that arrive in our centre with an image and histological confirmed diagnose of early BC and an US exam that shows high probability of node affection will be considered as potential candidates. Although by that time some inclusion criteria, such as undergoing NACT, will be unknown, they still will be considered as potential candidates, and so they will be informed about the study as well as receiving, reading and signing the informed consents. If they agree to participate a clip may be placed in the pathological node tested by US.
As some patients can initially take part in the study but will not meet some of the required by the trial inclusion criteria afterwards, they will be explained that there exists the possibility that they are ultimately not able to participate in the study as it is crucial and essential that all inclusion criteria are met by each of the patients. Criteria such as having a pathological node positive status or being a candidate for NACT can only be assessed after the time of the recruitment when a clip is placed, and so there is the possibility to be initially included as a potential candidate and having to be excluded afterwards for not meeting all inclusion criteria.

Sample size

The GRAMO program was used to calculate our sample size.
Accepting an alpha risk of 0,05 a beta risk of 0.2 in a bilateral contrast, we need **110 patients**, 55 in each group, if we want to recognize a statistically significant **difference** of **6%** or greater between the FNR. The common standard deviation we assumed is 10%. Due to our own sample recruitment characteristics, we have anticipated a **sample loss of 20%**. In our recruitment process we have estimated that an important percentage of patients will not meet all criteria requires at the end, even if they did meet them at the beginning of the trial.

Time of recruitment

According to the sample size calculation we need 110 patients. According to the data provided by the Unit of Mammary Pathology Department, every month at the *Hospital Universitari Doctor Josep Trueta* are diagnosed and operated a mean of 5 patients that would meet the inclusion criteria. So a total of 60 patients are operated every year. Considering these numbers, we will need about **two years** to recruit the sample.

Randomisation methods and masking techniques

After the patient recruitment we will do a randomisation in order to avoid the selection bias, placing them to one of the two groups of intervention, [Clip] or [No clip].

Before beginning the interventions, the investigators will randomly decide which intervention is performed to every group. The patients will be placed in the [Clip] group or the [No Clip] group with a simple 1:1 randomisation. As the patient group must be blind, they will not know in which group they will be placed.

7.4 VARIABLES AND MEASUREMENTS

7.4.1 Independent variable

Independent variable is represented by the **placement of a clip in a node**. The variable is a qualitative nominal variable and is to be allocated in the aspiration needle puncture of the node (ANP) followed by the placement of a clip group or the ANP without the placement of a clip group.

7.4.2 Main dependent variable

The main dependent variable is the **false negative rate** and will be defined as (38):

$$\frac{\text{False negative events}}{\text{Total number of pathologically node positive patients}} \times 100$$

A False negative event will be defined as a case where the specified node (either the clipped node or the SLN) did not show metastases even though residual disease was seen in other axillary nodes. Number of patients that having a pathological positive status, both the clip node or SLN node were negative for presence of metastases.
It will be a quantitative continuous variable expressed in percentage.

7.4.3 Secondary dependent variables

In addition to the main dependent variable we will measure and register the following secondary dependent variables that are mainly included in the secondary objectives of this study. Once we obtain the results, they will allow us to have further information about the accuracy of the procedure of placing a clip.

- **Sensitivity** and **Specificity** of the SLNB with a clip after NACT procedure. Sensitivity will measure the proportion of pathological positive nodes that are correctly identified as such. It will be defined as:

  $$\text{*Sensitivity} = \frac{\text{Number of true positive (TP) pathological nodes}}{\text{Number of positive nodes [TP + FN]}} \times 100$$

  Specificity will measure the proportion of pathological negative nodes that are correctly identified as such. It will be defined as:

  $$\text{*Specificity} = \frac{\text{Number of true negative (TN) pathological nodes}}{\text{Number of negative nodes [TN + False positive (FP)]}} \times 100$$

  TP, FN and FP will be obtained with the data of the pathological examination at the end of the study.
  Both Sensitivity and Specificity are quantitative continuous variables and will be expressed in percentage.

- The **percentage of patients that reach** a pathologic node negative status (**pCR**) **after NACT**. It will be defined as:

  $$\frac{\text{Number of patients with pathologic negative status}}{\text{Total number of patients that undergo NACT}} \times 100$$

The number of patients with pathologic negative status will be determined with the pathological examination of the sample nodes excised from the ALND procedure. The pCR will be defined as a grade 5 in the **Miller-Payne** histological scoring system (table **X**). It is important to clarify that, even we will be calculating the focusing on the pathologic status of the nodes, a pCR will be considered when the patient has a pathologic negative status and also has a pCR in the breast tumour.
It will be a quantitative continuous variable and it will be expressed in percentage.

- The **percentage of patients** where the **clipped node is not identified as a SLN**. It will be tested in both patients with pathologic negative status and pathological positive status among the participants of the group where a clip has been placed. It will be defined as:

$$\frac{\text{Nº of patients with discordance between the clipped node and SLN}}{\text{Total number of patients that the ALND is performed in the clip node group}} \times 100$$

It will be a quantitative continuous variable and it will be expressed in percentage.

7.4.4 Covariates

The covariate variables are factors that are not part of the main experimental manipulation but they are clinic and pathological features of the sample and the study population and so have an effect on the dependent variable. In a randomized clinical trial, the presence of covariate does not require any statistical adjustment because they will be distributed randomly in the large sample. However, they will be mentioned for further classification (table 22):

- **Age** (years): Qualitative dichotomous variable. Distributed into 2 groups: [>= 50] and [>50]

- **BMI** (kg/m^2): Qualitative dichotomous variable. Distributed into 2 groups: [≤ 25] and [>25]
- **Location of the main lesion**: Qualitative variable. Distributed into 8 groups: [Upper outer quadrant], [Upper inner quadrant], [Upper central area], [Mediocentral area], [Lower outer quadrant], [Lower inner quadrant], [Lower central area] and [Subareolar area]
- **Clinical tumour stage at diagnosis**: Qualitative variable. Distributed into 3 groups: [cT1], [cT2] and [cT3].
- **Clinical node stage at diagnosis**: Qualitative variable. Distributed into 3 groups: [cN1], [cN2] and [cN3]
- **Molecular phenotype**: Qualitative variable. Distributed into 4 groups: [HER2+] ,[TNBC], [Luminal A] and [Luminal B]
- **NACT regime**: Qualitative dichotomous variable. Distributed into 2 groups: [No use of trastuzumab] and [Use of trastuzumab]

Table 22: Covariates

Age (years)	**Clinical node stage at diagnosis**
>= 50	cN1
<50	cN2
BMI (kg/m^2)	cN3
<=25	**Molecular phenotype**
>25	HER2+
Location of the main lesion	TNBC
Upper outer quadrant	Luminal A
Upper inner quadrant	Luminal B
Upper central area	**NACT regime**
Mediocentral area	Trastuzumab
Lower outer quadrant	No Trastuzumab
Lower inner quadrant	**Clinical tumour stage at diagnosis**
Lower central area	cT1
Subareolar area	cT2
	cT3

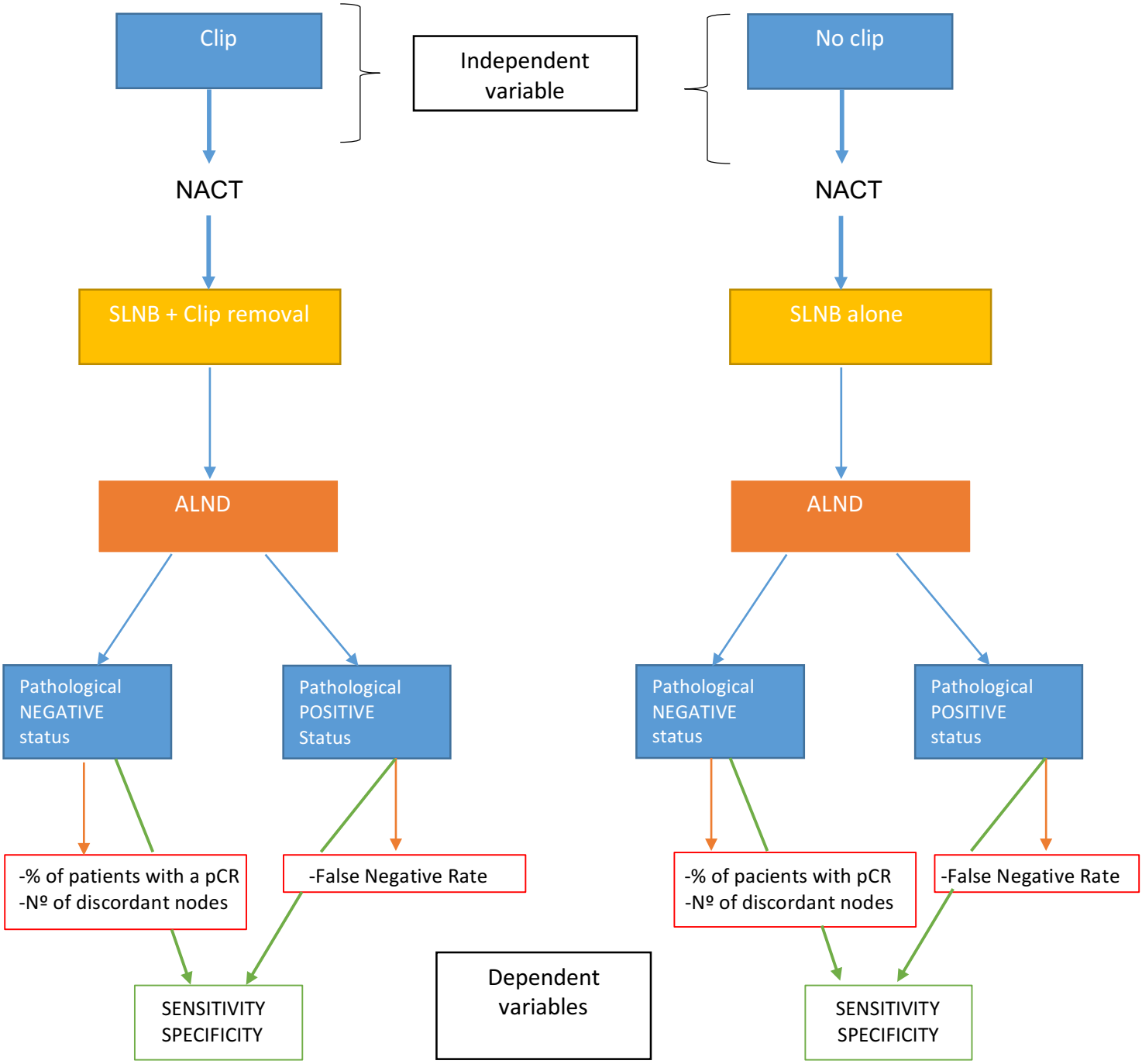

Figure 4: Study design and variables.

7.5 DATA COLLECTION AND PROCEDURES

Patients who have undergone an ANP and will potentially join this trial, previously have to be evaluated by several professionals of a multidisciplinary team including oncologists, gynaecologists, surgeons, nuclear physicians, radiologists and anaesthesiologists. Each patient's case will be exposed and discussed in our Hospital Breast Cancer Committee at least two times during the trial (figure 5).

7.5.1 First trial visit

The patient will arrive at our department with a diagnose of a breast tumour with the results of the pathological exam of this tumour. An US exam will be done to assess the node status. If the US exam shows with a high probability a node invasion (a N1 state) the patient will be considered as a potential candidate.

Therefore, the patient will be explained the possibility to take part in the study. An information sheet containing all the information about our study will be given (ANNEX 1). The patient will also receive the informed consent and it will be discussed if there are any doubts about the involvement in the trial. If the patient agrees, the informed consent to participate in the clinical trial will be signed (ANNEX 2)

Immediately after the US exam, the Radiologist will perform an ANP as well as randomly placing a clip node.

In the first visit the tumour molecular subtype, the BMI and the location of the main lesion will be collected from every patient.

7.5.2 Histological examination of the pathologic node

The material obtained by ANP from the node will be processed and histologically examined to determine if there exist node metastases.

By this time the node pathological status will be collected and only the patients that after the exam have a citology-proven positive pathological node status will be included in the trial, even if they were recruited before.

7.5.3 Radiological exam of disease extension

In all patients that have been diagnosed with a BC, there needs to be examined the possible existence of distant metastases. The assessment of metastasic invasion will be done by toraco-abdomino-pelvic **CT** and a **Bone scan**. Patients will need to sign the standard informed consent for the radiological procedures. Patients showing presence of distance metastases in the one of the two radiologic exams will be excluded from the trial.

7.5.4 Case exposition to the Breast Cancer Committee

Before initiating NACT, the case has to be discussed in a multidisciplinary committee integrated by Radiologists, Surgeons, Gynaecologists and Oncologists, all experts in mammary pathology. If the Committee agrees that the patient is eligible and will benefit from a NACT regime, she will continue to take part in the trial.

7.5.5 Second trial visit

The patient will need to visit with the breast oncologist to schedule the NACT regime after it has been approved the individual recommendation for NACT in each case. The specific NACT regime will be collected as data. The field-expert oncologist will be the responsible for organising the NACT. The length of the NACT will be approximately 6 months. During this period, the oncologist will visit the patient every 2 or 3 months to evaluate the patient tolerance and to assess if there should be done any adjustments. The patient will have to signed the informed consent to authorize the NACT procedure (ANNEX 4).

7.5.6 Radiological exam of the NACT response

If the patients have been able to complete a full NACT regime, a MRI will be done to assess the quantitative tumour and node response to the NACT. It will be both assessed the tumour response and the axillary node response. The information will be presented again at the Breast Cancer Committee to recommend the surgery and decide which type of surgery should be performed depending on the MRI results.

7.5.7 2nd case exposition to the Breast Cancer Committee

The Breast Cancer Committee will decide the eligibility for a SLNB and which type of breast surgery (tumorectomy or mastectomy) should be done, considering the data from the radiological status of the tumour and nodes.
If the patient is eligible for both SLNB and surgical treatment, she will continue in the study. Between the completion of the NACT and the surgery there has to be a time period of minimum a month.

7.5.8 Preoperative visit

- **Gynaecologist Department**: Between 15 and 30 days before the surgery the patient will have a visit for the three surgical procedures; the SLNB, tumour resection and the ALND. The patient will be informed about the 3 procedures and the informed consent of the three procedures will be signed by the patient (ANNEX 3). The time of hospital admission will be scheduled considering the need to inject the radiocolloid some hours before entering the surgery.

- **Anaesthesiology Department**: The patient will have a visit at the Anaesthesiology Department for the preoperative assessment. Some exams will be required: blood test, electrocardiogram and a thorax X-rays. American Society of Anaesthesiologists (ASA) Physical Status Classification System score will be used for surgical risk. They will sign the anaesthesiologist informed consent for the intervention

7.5.9 Surgery

The patient will undergo at a one-time surgery the 3 procedures:

1.Surgical tumour resection: The surgical resection will be a mastectomy or a tumorectomy depending on the decision previously established by the Committee.
2.SLNB and clip node removal: A SLNB will be performed to all patients with the removal of the identified SLNs. In the group where a clip has

been placed, the detection of the clip will be done by US, as well as the assurance of its removal. The SLNs together with the clip nodes of every patient will be sent to histologic exam. The result of the pathologic node status of the SLNs will be known intra operatory and so it will be collected how many SLNs were excised to each of the patients, and also in how many patients the nodes detected as SLNs were not the clip nodes. The SLNB will be performed by a trained surgeon and a nuclear physician. In those patients where it has not been able to identify any SLN by SLNB will be considered as a loss and will not be counted when assessing the FNR and other calculations.

3. ALND: Immediately after performing the SLNB a standard ALND will be performed in all the clinical trial patients. The standard ALND is from level I to level III. The ALND will be necessary in order to obtain the pathologic status of the nodes that haven't been excised during the SLNB, and are precisely the examination of all these nodes that will give the real and pathological node status. While the ALND is being done, the removal of the clip node must be again assured. The ALND will be performed by a trained surgeon.

7.5.10 Hospitalization

After the surgery patients will go to *Unitat de Reanimació Assistida* (REA). If there is a correct evolution, patients will be admitted to Gynaecology Department. Between the 2nd and 3rd postoperative day, if there is a positive evolution, patients will receive the hospital discharge report. After the surgery the patients will not need to be followed and so the data collection will be considered to be finalised.

7.5.11 Pathological examination of the nodes excised at SLNB and ALND

It will be crucial for the study that these procedures are done with the highest possible accuracy, as it is the data that will ultimately allows to calculate the false negative rate of the procedure. It will be of extreme importance that each patient's pathologic result is her actual and own pathologic result and that

samples are not switched during the node sample collection, transportation and examination.

The pathological exam will be performed by a well-trained pathologist.

The Miller-Payne scoring system will be used to express the pathological status.

From each patient we will collect:

- ✓ **Pathological status** of the **SLNs** excised by SLNB.
- ✓ **Pathological status** of the **clipped nodes** excised (in the group with a clip in the node).
- ✓ **Pathological status** of all the **other nodes** excised by ALND.

7.5.12 Pathological data collection and calculations

Once all the pathological examinations have been done individually, they should be collected into a common database to calculate the **FNR**, **Sensitivity**, **Specificity**, **%** of patients with **nodal pCR** and the **%** of patients where there exists **discordance** between the **SLNs and the clipped nodes** (figure 4)

- **FNR**: Once each of the two groups is subdivided into pathological positive status and pathological negative status, we will select the patients that showed a pathological positive status and we will calculate how many of them had a negative pathologic status in the SLNs and/or clip nodes and still there exists residual disease in nodes not being identified as SLNs or clipped nodes. This last subgroup of patients will represent the FN events. To calculate the FNR of every type of SLNB, we will divide the FN event of each group by the total patients that had a pathological positive status. It will be expressed in percentage and separately in the two different groups.

- **Sensitivity:** Sensitivity will be calculated as it has been mentioned before. It will be expressed in percentage. Sensitivity quantifies the ability of the procedure to avoid the false negatives, so true pathological positive nodes are not missed. As the aim of the study is to determine if placing a clip will improve the accuracy of the results from the SLNB

procedure, we will calculate the Sensitivity in the group with a node clipped and in the group without the clip separately.

- **Specificity**: Specificity will be calculated as it has been mentioned before. It will be also expressed in percentage. Specificity quantifies the ability of the procedure to avoid the false positives, so pathological positive results really represent the presence of node metastases and not a mistaken result. As well as for the Sensitivity, it will be calculated in both groups of the study separately.

- **% of patients with nodal pCR**: Within each of the two interventional groups (clip and not clip), we will split the groups in two; the ones that have reached a nodal pCR (pathological negative status) and the ones that still do not have a pCR (Miller-Payne Grade 1-4). The number of patients that show a pathological negative status will be divided by the total number of patients within the group. It will be expressed in percentage and separately in the SLNB with clip and in the SLNB alone.

- **% of the patients with discordance between the identified SLNs and the clipped nodes**: This variable will be calculated only on the SLNB with clip removal group. We will calculate the number of patients where the SLNs retrieved where not including the clipped nodes. It will be calculated in both pathological negative status group and pathological positive status group. This total number of patients where there exists discordance will be divided by the total number of patients which a clipped was placed at the time of the Aspiration Needle Puncture (ANP) procedure.

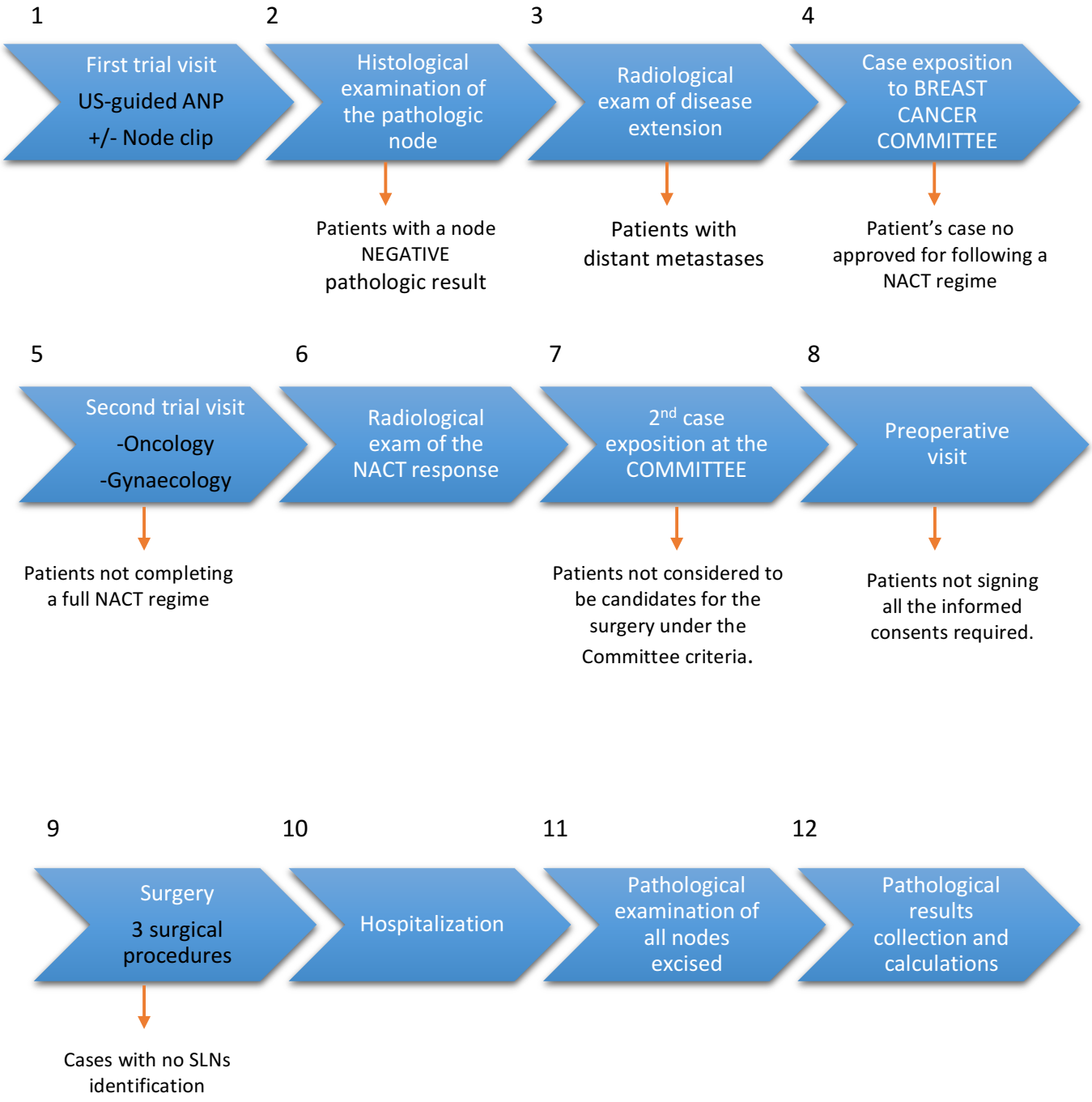

Figure 5. Diagram showing the procedures and data collection chronologically. Red arrows show the patients who would be excluded in every step of the trial.

7.6 INTERVENTIONS AND PROCEDURES

7.6.1 Placement of a clip

During the ANP US-guided procedure the Radiologist will randomly place a clip to the pathologic node. The clip will be similar as the ones they place around the tumour biopsy area. In our centre there is a Mammary Pathology Unit integrated by various specialists such as an expert-field Radiologist. Radiologists are well trained to perform clipping procedures in breast tumours and so the technique to put a clip in the node will be similar to the one they use with the primary breast tumour.

7.6.2 SLNB and clip removal

The process involves the administration of a **radiopharmaceutica**l in the breast followed by its detection through **imaging** by a **gamma-probe** (11)(5), (8), (13). The SLNB procedure follows different steps (Figure 6)

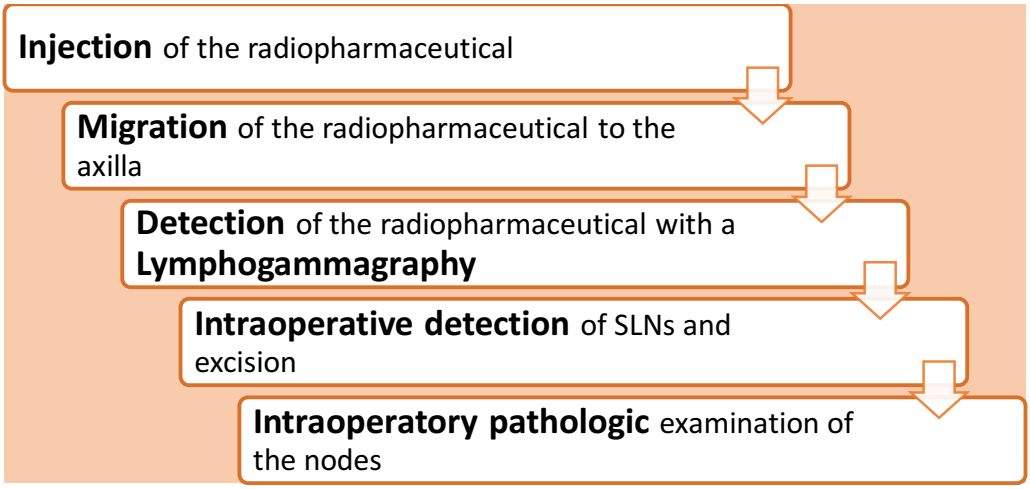

Figure 6. Steps of the SLNB procedure

- **INJECTION**

First, after anaesthetizing the patient, the radiopharmaceutical is injected and should ideally migrate from the injection site and rapidly accumulate within the SLN without substantial spill over. There is no consensus about the most appropriate technique regarding the site of injection. The most common routes are **peritumoral, intratumora**l and **intradermal** (Figure 7). However, the **periareolar** or **subdermal** injection could also be options to consider (13).

Some studies show that the detection rate is improved by intradermal injection of the tracer, however, there are no differences between the false-negative rates between the different techniques (5). On the other hand, some studies have been unable to determine if there is significant difference in the successful rate and have determined that the detection rate is independent of the injection site (6).

The main drawback of the intradermal injection is that as it is superficial it would rarely demonstrate internal mammary nodes. For **non-palpable lesions** intra or **peritumoral** injection would be the best approach. So if an internal mammary drainage is suspected, a deeper intra or peritumoural injection technique is recommended. For **lesions** that have been **already extirpated** or are anatomically close to the axillar area, it is recommended to use the **periareolar** or **intradermal i**njection of the radiotracer (5,13).

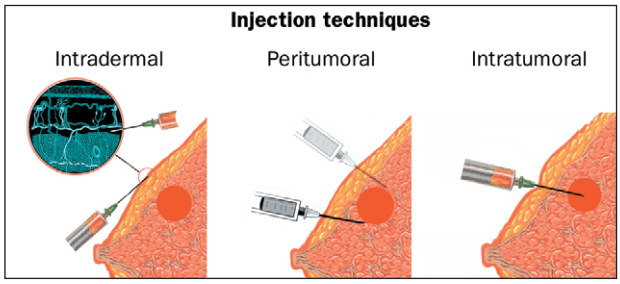

Figure 7. Various techniques for delivery of the radiopharmaceutical (5)

- **MAPPING AGENTS**

The existent standard SLNB method is a **dual technique** involving injection of the radiopharmaceutical that contains a **technetium-labelled radiocolloid** and **blue dye**. However, nowadays clinical guides consider the **technetium-labelled radiocolloid** as the **imperative technique**. It is also acceptable to use the dual technique (radiocolloid with blue dye), especially in learning phase of the procedure. The individual use of blue dye is nowadays contraindicated (8,13).

The **radiocolloid**, is usually an albumin colloid in Europe. In Spain, the recommended tracers are the albumin nanocolloid, the tin colloid and the sulphide colloid (13). Each of them should be used according to patient characteristics and its availability at the health center. It offers several advantages as it efficiently trapes in the sentinel node, while the other labelled

ligands pass typically pass into second echelon nodes. Radiocolloids enable pre-operative sentinel node imaging, and facilitate rapid and easy intraoperative identification by the surgeon using a gamma probe. Pre-operative imaging identifies the draining lymphatic basin and number of sentinel nodes. This is helpful when sentinel nodes lie in unusual locations such as internal mammary nodes. The identification of a sentinel node on pre-operative imaging is highly predictive of the success of the subsequent surgical procedure. Although it provides the surgeon with a road map, the precise anatomical location of the node can only be determined using intra-operative gamma probe. The radiocolloid labels the radiotracer. The **universal radiotracer** used is **Technetium-99m**. It offers several advantages It is a pure gamma radiation emitter, hence offers excellent tissue penetration and it is safer compared with alpha and beta particles. It is as well cheap and available in every nuclear medicine department (5,13).

Options of dye include lymphazurin blue, isosulphan blue, methylene blue, indocyanine green and indocarmine. The injection of blue dye enables the surgeon to identify blue-stained lymphatic tracts draining from the tumour. Following these tracts allows identification of the first draining lymph node. The recommended dosage and is 2ml and should be injected between 10 and 15 minutes before the intervention procedure. If a blue dye is used, it is also recommended to massage the injection site immediately after injection (5,6,13). It is generally recommended to use both techniques combined as it provides greatest identification rates of the sentinel lymph node. It carries many advantages. The **identification rate** of the sentinel lymph node is increased to **96-100%** when **both techniques are used** simultaneously, compared to an identification rate of 86-90% when single agents are used. It also reduces false negative rates, being lower than 5% and enables the identification of sentinel nodes in unusual locations. If one technique fails, the other may succeed (5,6,13).

- **LYMPHOGAMMAGRAPHY**

During the post injection period it is imperative to use a lymphogammagraphy to make sure that the radiotracer has **indeed migrated** and to **demonstrate** possible **extra axillar drains**. In case there is no migration or axillar drainage visualised, it is recommended to reinject the radiotracer in the same day. The lymphogammagraphy should be done as close as possible as the intervention moment. In case that limphogammagraphy fails to identify the SLNs, if there is available a SPECT-CT equipment, it is recommended to use it (8,13).

- **INTRAOPERATIVE DETECTION**

As it is a surgical procedure a signed informed consent by the patient is required. Regarding the anaesthesia, both general and local anaesthesia with sedation are accepted. After the procedure a manual examination should be performed to make sure there are no remaining nodes that could be potentially candidates to perform a biopsy. It is also recommended the intraoperative use of a gamma probe, especially in cases where a difficult identification of the SLN is expected (13).

It has been concluded that there should be **no absolute upper threshold for the number of the SLNs** that need to undergo biopsy. The sampling of additional node carries only a small increase increased risk of morbidity, but may alter treatment conditions considerably. The ability to identify multiple SLNs, when they exist, improves diagnostic accuracy of SLNB. Overall, it is **acceptable** to sample **3 LNs**, but is **recommended** to sample only **2** (5,13).

- **PATHOLOGICAL EXAMINATION**

The detection and extraction of the lymph nodes is followed by an **histo pathological examination** of the sentinel lymph node. The diagnosis of the SLN should be, when possible, **intra operatory**, in order to increase the technique's efficiency. The SLN status can also be assessed by molecular techniques. One-step nucleic acid amplification [**OSNA**] assay is a molecular procedure that can identify deposits of RNA breast cancer cells in the SLN, and has a higher sensitivity and specificity than histological techniques. OSNA assay also allows to avoid the important false negative rate of the intraoperative histological study (5–7,13,40).

8 STATISTICAL ANALYSIS

Immediately after the results from the pathologic exam the statistical analysis will be done. The Statistical Package for the Social Sciences (**SPSS**)for Windows® will be used.

Univariate analysis: The results will be expressed as percentages for categorical variables, and as mean +/- standard deviation or mean for quantitative variables depending on whether or not they are normally distributed.

Bivariate analysis: For quantitative continuous variables we will use the Kolmogorov-Smirnov test to see whether or not they are normally distributed. Depending on these results, the T-Student test or the non-parametric Mann-Whitney test will be used.
For categorical ordinal and categorical nominal variables we will use the Chi-Square test.
A confidence interval of 95% will be assumed and a $p<0.05$ will be considered to be statistically significant.

As it is a randomized clinical trial type of study, where the covariates have been randomly distributed, we do not expect to perform any multivariate analysis. In such case, we would use a logistic regression model.

9 WORK PLAN AND CHRONOGRAM

The study is designed to last **3 years** and **4 months**, from January 2018 to April 2021, and it has been designed to be carried out in **5 phases** (figure 8).

9.1.Preparation (3 months)

During this phase, the study will be elaborated and the protocol will be written. All the specialists from the Mammary Pathology Unit of our hospital will participate in the creation of the trial. Then, the protocol will be studied and evaluated at the Clinical Research Ethics Committee (CEIC) of the Hospital *Universitari Josep Trueta*. We will wait for its approval.

9.2 Coordination (1 month)

After the approval the designated main researcher will meet all the other researchers, including surgeons, radiologists, pathologists, oncologists, gynaecologists and the statistic expert, as well as all the other administrative staff that will have a roll in the study. In the first meeting the aims of the trial, the methods and the design of the study will be exposed and discussed. Once all the researchers agree to participate and have exposed their ideas, detailed instructions to the different researchers will be given. As for the patient recruitment, the clinical research associate will be the one to coordinate and explain the recruitment to the rest of the team. The study team will be in constant touch during all the trial and a meeting will be set in 3 months to control and assess the study progression.

9.3.Field work and data collection (24 months)

As it is a consecutive recruitment type of study, the sample recruitment and the interventions period will be done at the same time. And as our study does not include a follow-up period after the surgery because al the data will be collected during the interventions, the data collection will also be done simultaneously as the sample recruitment and interventions.

- Sample recruitment: (24 months) All patients meeting the inclusion criteria will be included in the study and will be randomly placed in one of the two groups of intervention. Since we need 110 patients and in Hospital *Universitari Josep Trueta* we have estimated that if our department operates 5 patients per month, the sample recruitment will last 24 months (5 patients/ month and we need 110 patients)
- Interventions (24 months): We will perform the interventions during the 24 months.
- Data collection (24 months): Data collection will be done during two of the interventions; the SLNB and the ALND. The most important and crucial part of our data collection is the pathological examination. This will be carried out during the 24 months we will be doing the interventions.

9.4.Data analysis and interpretation (5 months)

After all medical procedures are done and results of the pathologic examination of the data collected are obtained, the data will be entered in a data base and the statistical analysis expert of the trial will perform the analysis. The results will be materialized in the article that the trial team will write. After the article is reviewed it will be ready to be published.

9.5.Publication and dissemination (7 months)

The article will be published in scientific journals and the researchers will inscribe and assist to national and international congresses to expand the trial results among the different medical societies.

TASKS	2018			2019	2020			2021
	Jan - Mar	Apr	May - Dec		Jan - Apr	May - Oct	Nov - Dec	Jan - Apr
Protocol Preparation								
Coordination								
Sample recruitment								
Interventions								
Data Collection								
Data Analysis								
Result Interpretation								
Publication								

Figure 8. Chronogram

10 ETHICAL AND LEGAL ASPECTS

The study will involve humans and must respect the principles of the Helsinki Declaration from 1964. Therefore, before starting this trial, the study protocol will have to be evaluated and approved by the Clinical Research Ethics Committee (CEIC) of the Hospital *Universitari Josep Trueta de Girona* to ensure that the trial is regarding, following and respecting the ethical principles included in the Helsinki Declaration. If the CEIC gives further ethical indications they will also have to be followed and respected. In addition to the CEIC, the management board of our centre will have to approve the trial as well. Once approved, the trial will be registered in ClinicalTrials.gov and EudraCT in order to avoid publication bias.

The protocol will follow the Spanish law *Ley 14/2007, del 3 de Julio, de Investigación Biomédica* as our trial involves invasive procedures. Moreover, the privacy of the participants and personal information will have to be protected according to the Spanish law *Ley Orgánica 15/1999, del 13 de Diciembre, de Protección de Datos de Caràcter Personal* to ensure the confidentiality of the participants medical data.

All the Beauchamp and Childress bioethical principles will be respected. The principle of autonomy will be respected by informing the participants about the interventions and procedures of the study providing them with an information document (ANNEX 1). All the possible doubts will be answered. For the patients to express accordance they have to sign the informed consents for the procedures that entail participating in the trial (ANNEX 2 and 3). Patients will be able to leave the study and revoke her informed consent at any time. Secondly, to respect the beneficence and non-maleficence principles, the study counts with exclusion criteria in order to avoid performing a procedure to a patient when it is not indicated and will not bring any benefit. Finally, to respect the justice principle all patients will receive the same conditions and will be equally treated. To achieve this principle every participant will remain anonymous and will be randomly placed to one of the different groups without any differences.

11 STRENGTHS AND LIMITATIONS

The main limitation of this study is that the design is a simple-blinded instead of a double blinded that will imply more accuracy measured by the Oxford Quality Score or Jadad Scale (41). There is no possible way that the study would be designed as a double-blinded, as the interventional radiologists that places the clip and the surgeon that has to remove it afterwards, can not be blind. To reduce this detection bias, the patient, the pathologist and the statistical expert will be all blind.

Another limitation of the study is related to the recruitment. A consecutive non-probabilistic recruitment will be done and there is the risk of not obtaining the most representative population, and this could cause a selection bias. We estimate that the reference population is very similar to our sample. To minimize the possible selection bias, very extensive inclusion criteria have been set as well as well as exclusion criteria. This will help to have a very similar sample to the population of study and at the same time we will reduce the confusing factors.

Another limitation of the study comes from the possibility of losing, misplacing or switching histological samples. Each patient's pathological exams that are relevant for this study will be performed in two times; at the time of the SLNB and at the time of the ALND. From each patient we will collect more than one data and the data from both procedures will be ultimately joined together to do the calculations needed to obtain the dependent variables. This method to obtain biopsy samples in two different times, followed by the unification of the data of the results of each of the two samples to calculate them all together at the end, entails an inherent risk of misplacing, switching and losing samples and data of the study. We attempted to minimize the possibilities of such events, by collecting the status of the SLNs at the time of the SLNB intraoperatively. With this, we will get every patient's first result immediately and the possibility of losing or misplacing samples will be very difficult to happen at the time of SLNB.

This study will be unicentric. To do unicentric study carries many strengths and limitations. One of its limitations is that as the patients will only be recruited in one centre, meaning that both recruitment and interventional periods will be longer. But on the other hand, we will be less procedure bias between the interventors. As in our study the data analysis it will be carried out mainly by pathologists, a unicentric study will avoid possible discrepancies of examination of the samples between different pathologists.

The main strength of our trial is the randomisation when distributing the patients into two groups, which will create a symmetrical distribution. This symmetrical distribution allows the extrapolation of the future results on the general population.
Another important strength is the short time period estimated to conclude the data collection. As it is a trial implies a design were the data collection is done in a very short time since the patient enters in the trial until the last data is obtained, the chances of sample loss are very reduced as there is no follow-up period and there are almost no possibilities of patient loss for such reasons.

In addition, we also have considered that the design of this study makes its realization very feasible. We consider that the study we propose would have a strong feasibility for several reasons. In the first place, this trial will take place exclusively at our centre, where we will provide everything that is necessary from the beginning of the study until the end. The patient visits, operating rooms, NACT regime, the hospitalization cares costs and the involved surgeons, radiologists, nuclear physicians, pathologists and oncologists salaries, will all be covered by the hospital. Secondly, for the extra SLNB procedure that all patients need to undergo, that appears in the budget, it will be carried out in the same hospital previously setting an agreement of payment. Our centre performs SLNB procedures in the daily practice and so it will be done in our hospital, to make sure the data collected from the SLNB stays in our hospital for further examination, and at the same time is done by the same specialists that will perform the rest of the interventions from the trial. In third place, in our centre we count with a Unit of Mammary Pathology, that is composed of several specialized physicians, being a multidisciplinary unit. This

means that all the doctors from different specializations work with mammary pathology in their daily practice. This allows to assure the reliability and quality of all the interventions, the possibility to meet several times during the trial in the multidisciplinary Breast Cancer Committee of our hospital, and that all t specialists will be in constant contact. It will also not entail any extra costs for medical formation. Last but not least, we would like to highlight that our centre treats patients with the same characteristics of the ones in our study, so we will be able to use the same circuit the patients with operable breast cancer normally follows. This makes the study very feasible, avoiding problems of coordination, organisation and payment.

12 BUDGET

For the realization of this study we will need an inversion of **68,995.0€**
Performing this clinical trial will entail an increase of the cost of al procedures. While the majority of the interventions the participants will have to undergo will be covered by our National Health System in our centre, some other interventions will not be included and will have to be considered in the budget.

As all patients diagnosed with early breast cancer follow up the same clinical interventional steps we propose, most of the interventions will not entail extra costs. Almost all procedures such as radiological examinations, visits, NACT regimes, tumour resection and ALND will suppose no extra expenses as they are the same interventions for any breast cancer patient in our centre. Any extra cost in medical formation of the physicians is expected, as our Radiologists, Surgeons and expert pathologists perform all the interventions in their daily practice. As for the hospitalization, the interventions we propose neither will entail extra days of hospitalization, nor an increase in the budget.

Even if in our centre and our National Health System covers the costs of the SLNB procedure, the case of our patients is still not officially recommended as a procedure. Thus, we have assumed that it will suppose an extra cost. The **expanses of SLNB** procedure will regard either the cost of the procedure itself as well as the posterior histological examination. We have estimated that each SLNB will have a cost of **500€** approximately.
As we are working with invasive procedures it is necessary to take out an **insurance** for each patient, with a total of **1100€** (110 patients for 100€/patient)
A **statistical expert** for the randomisation of the patients and the data anlysis is also needed. We assume that there will be 80 hours of work with a salary of 25€/hour, it will entail an extra expense of **2000€.**
A part from the statistical expert we will also need a **clinical research associate** who will be responsible for the data monitoring and control, giving assessment and coordinating the medical staff involved as well as the patients.

We expect there will be approximately 200 hours of work at a salary of 25€/hours, with a total cost of **5000€.**

The **cost of the printing materials** for information sheets and informed consents regarding the trial, we have estimated a cost of 0,50€/patient, with a total cost of **55€** (0,5€ x 110 patients).

After we get all the results, a crucial part of our clinical trial is the publication and dissemination of our study. Publishing the study in the international scientific journals will imply a cost of 1500€ plus 600€ for the translation, a total of 2100€. As for the dissemination of our study we have planned to attend to a national congress and an international congress. For the national congress we have estimated a cost of 400€ and for the international congress we have estimated a cost of 1000€. During both congresses we have included the travel transportation, accommodation and food (1000€/person). The total **expenses** for the **publication** and **dissemination** will entail a cost of **5500€.**

Table 23. Budget

	Price	Quantity	Total
STAFF AND SERVICES			
Statistical expert	25€/h	80 hours	2000€
Clinical research associated	25€/h	200 hours	5000€
Meetings and formation	100€	3	300€
Insurance	100€	110 patients	1100€
MATERIAL AND PROCEDURES			
Informed consent printing	0,5€	110 patients	55€
SLNB	500€	110 patients	55.000€
PUBLICATION AND DISSEMINATION			
Publication expenses	2100€	1	2100€
Inscription to a national congress	400€	1	400€
Inscription to an international congress	1000€	1	1000€
Travel transportation, accommodation and food	1000€	2	2000€
TOTAL		**68,995€**	

13 IMPACT

Nowadays, breast cancer patients with proven positive-node metastases undergo ALND. All patients have to undergo ALND even if has been largely proved that receiving NACT before the tumour resection and ALND there is a possibility, in many cases, that there appears a pCR in both the tumour and the axillar nodes. A pathologic complete response (pCR) entails that there are no malignant cells identifiable in the bed tumour and in the axillar nodes after NACT. If many women reach a pCR in the breast tumour, nowadays, the proposed therapeutic surgical procedure is to perform tumourectomy, instead of a mastectomy. This change in the aggressiveness of the breast surgery has implied many cosmetic outcomes for many patients, as well as less need of further plastic reconstructions, that would mean a nuance for the patient as well as an inversion from the National Health System.

If our hypothesis is confirmed, the Sentinel lymph node biopsy (SLNB) of the clipped nodes after NACT, could be considered as an accurate and acceptable procedure, ready to be approved by our National Health System and implemented in the day-to-day therapeutic procedure of patients with Breast Cancer. If we could achieve its implementation, we would be able to offer our patients the possibility to improve their quality of life after surgery, offering them a less invasive axillar procedure, avoiding the many postoperative nuances and long-term side effects that an ALND carries. We will be offering an intention-to treat procedure that, at the same time, will offer better quality-of-life-after-surgery options.

If we confirm our hypothesis, this will also have a significant impact in the expenses that all these procedures generate, and the necessary investments from our Health National System. Avoiding an unnecessary ALND will not only benefit the patient's quality of life, it will also reduce the inversions in posterior physical therapy that most of the patients need to undergo to avoid the lymphedema caused by the ALND. If ALNDs are not performed, it will mean an

economic reduction, as the National Health System will be saving the standard costs of an ALND, that is a surgical procedure and implies costs.

So, with the results of this study we expect to improve the treatment of breast cancer, improving the overall quality of life after surgery of breast cancer patients, and especially with aggressive type of tumours that nowadays entail more aggressive surgical procedures and consequently worse quality of life after surgery. This will also suppose a significant reduction of the National Health Care expenses. Both improvements should be considered together with the fact that Breast Cancer is a disease with a great prevalence and the impact would happen on a large scale.

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15 ANNEXES

ANNEX 1

Hospital Universitari de Girona Doctor Josep Trueta

Generalitat de Catalunya
Departament de Salut

FULL D'INFORMACIÓ AL PACIENT SOBRE L'ESTUDI

"SELECTIVE EVALUATION OF CLIPPED NODES: IMPROVING AXILLARY EVALUATION AFTER NEOADJUVANT THERAPY IN NODE POSITIVE BREAST CANCER PATIENTS"

"AVALUACIÓ SELECTIVA DE GANGLIS CLIPATS: MILORANT L'AVALUACIÓ AXIL·LAR DESPRÉS DEL TRACTAMENT NEOADJUVANT EM PATIENTS AMB TUMOR DE MAMA AMB GANGLIS POSITIUS"

PROPÒSIT I OBJECTIU DE L'ESTUDI

El seu metge el convida a participar a l'estudi clínic coordinat per l'Hospital Universitari Josep Trueta, ja que compleix els requisits per a participar-hi. Aquest estudi consisteix en la comparació de dos procediments diagnòstics per a evaluar la resposta axil·lar després de la quimioteràpia.

Aquesta intervenció, anomenada biòpsia de gangli sentinella, consisteix en la detecció del gangli de l'axil·la anomenat sentinella i la seva posterior extracció per ser processat i examinat per anatomia patològica. D'aquesta manera, els professionals obtenim informació sobre com han respòs a la quimioteràpia aquells ganglis que estaven prèviament afectats per les cèl·lules del tumor. Existeixen variacions d'aquesta tècnica que consisteixen en col·locar un clip en el gangli en el moment en que es sospita que existeix un gangli afectat. La diferència entre posar un clip i no posar-lo, es que quan posem el clip , quan després de la quimioterapia volem evaluar si l'afectació dels ganglis ha desaparegut, ens assegurem que el gangli que estem analitzant era de ben segur el que estava prèviament afectat. Ens dóna una major seguretat de que el gangli que pot sortir negatiu en la biòpsia de gangli sentinella es el mateix gangli que prèviament es mostrava com a afectat.

Totes les pacients que entrin a formar part d'aquest estudi se'ls practicarà les intervencions que estan establertes avui en dia per al tractament del càncer de mama. Avui en dia l'afectació dels ganglis axil·lars confirmada per citologia abans de la quimioteràpia, implica que a la pacient se li ha de practicar un buidament de tots els ganglis de l'aixella. Aquest estudi implica que moments abans d'aquest buidament axil·lar a cada pacient se li realitzarà la biòpsia de gangli sentinella amb l'extracció d'un gangli. Posteriorment, a totes les pacients se'ls practicarà el buidament axil·lar total estàndard.

Amb aquest estudi es pretén conèixer si la col·locació d'un clip al gangli ens permet reduir el nombre de resultats falsament negatius, i poder convertir aquesta biòpsia de gangli sentinella post quimioterapia com una prova fiable i fidedigna per poder-la implantar en totes aquelles pacients que després de la quimioteràpia hagin assolit amb alta probabilitat un estatus negatiu per a afectació axil·lar del seu tumor de mama.

PROCEDIMENTS DE L'ESTUDI

Es realitzarà un estudi preoperatori estàndard per determinar que es pot realitzar la intervenció.

En el moment de la cirurgia es decidirà de forma aleatòria, amb un 50% de possibilitats de rebre cada tècnica, a quin grup formarà part. Al acabar la cirurgia els ganglis sentinella i tota la cadena de ganglis extirpada s'enviaran a anatomia patològica per al seu estudi.

L'estada hospitalària no s'espera que es prolongui.

INCONVENIENTS I BENEFICIS

El protocol de tractament de les pacients de l'estudi no variarà respecte al que reben totes les pacients del nostre centre per al mateix diagnòstic. Per tant, el vostre tractament no es veurà amenaçat ni alterat en cap moment. El temps estimat en que es duran a terme les vostres intervencions tampoc s'espera que es vegi incrementat, així com tampoc la vostra estada hospitalària o les visites als diferents especialistes.

PARTICIPACIÓ

La seva participació a l'estudi és totalment voluntària. Si decideix no participar la seva atenció mèdica no es veurà influenciada en cap nivell.

Si desitja abandonar l'estudi, en qualsevol moment, és lliure de fer-ho sense donar explicacions i sense que això afecti al seu tractament normal o a la qualitat de les cures que rebrà.

El seu metge també podrà retirar-lo de l'estudi en qualsevol moment. Aquesta situació es podria donar si durant el procés de l'estudi, vostè no compleix un dels requisits per a poder seguir l'estudi que impliquen tant canvis en la seva situació clínica com també si no compleix amb el pla establert per l'estudi.

Se la mantindrà infomada de qualsevol nova informació disponible o que pugui afectar a la seva decisió.

Aquest estudi ha estat analitzat i aprovat per el Comitè Ètic d'Investigació de l'Hospital Universitari Doctor Josep Trueta de Girona, que ha dictaminat que és ètic i que amb els resultats publicats fins al moment en cap moment se'l pot perjudicar, ni a vostè ni a la seva salut.

ANNEX 2

Hospital Universitari de Girona Doctor Josep Trueta

Generalitat de Catalunya
Departament de Salut

FORMULARI DE CONSENTIMENT INFORMAT DE PARTICIPACIÓ A L'ESTUDI DEL PACIENT

CONSENTIMENT ESCRIT

TÍTOL DE L'ESTUDI: *"SELECTIVE EVALUATION OF CLIPPED NODES: IMPROVING AXILLARY EVALUATION AFTER NEOADJUVANT THERAPY IN NODE POSITIVE BREAST CANCER PATIENTS"*

"AVALUACIÓ SELECTIVA DE GANGLIS CLIPATS: MILORANT L'AVALUACIÓ AXIL·LAR DESPRÉS DEL TRACTAMENT NEOADJUVANT EM PATIENTS AMB TUMOR DE MAMA AMB GANGLIS POSITIUS"

Jo, _____, amb DNI _____:
He parlat amb el Dr/Dra _____

He llegit el full d'informació que se m'ha entregat
He pogut fer preguntes sobre l'estudi i s'han respòs de manera satisfactòria
He rebut suficient informació sobre l'estudi

Comprenc que la meva participació és voluntària
Comprenc que puc retirar-me de l'estudi:

- En qualsevol moment
- Sense donar explicacions
- Sense repercussions en la meva assistència mèdica.

En conseqüència dono lliurement el meu consentiment per entrar en aquest estudi

Signatura participant	Signatura Investigador/Metge
Data: _ _ / _ _ / _ _ _ _	Data: _ _ / _ _ / _ _ _ _

ANNEX 3

Hospital Universitari de Girona Doctor Josep Trueta

Generalitat de Catalunya
Departament de Salut

CONSENTIMENT INFORMAT PER A PROCEDIMENTS QUIRÚRGICS I/O TERAPÈUTICS

PACIENT

Jo_____he llegit el full informatiu que m'ha entregat el Dr LUIS MIGUEL ALONSO RUANO. He comprès les explicacions que m'ha facilitat, i el metge que m'ha atès m'ha permès totes les observacions I m'ha aclarit tots els dubtes i preguntes que li he plantejat. També comprenc que, en qualsevol moment i sense necessitate de donar cap explicació, puc revocar el consentiment que ara presto. Per això, manifesto que em consider satisfet/a amb la informació rebuda i que comprenc la indicació i els **riscs més freqüents** que poden aparèixer a nivell general durant la realització d'aquest **procediment quirúrgic i/o terapèutic així** com els riscos concrets que poden aparèixer en el meu cas donada la meva situació clínica i les meves circumstàncies personals (riscs personalitzats) que són:

_____.

REPRESENTANT LEGAL

Jo_____ en qualitat de_____del/de la pacient
He llegit el full informatiu que m'ha entregat el/la Dr. LUIS MIGUEL ALONSO RUANO. He comprès les explicacions que m'han facilitat, i el metge que m'ha atès m'ha permès totes les observacions i m'ha aclarit tots els dubtes I preguntes que li he plantejat. També comprenc que, en qualsevol moment I sense necessitat de donar cap explicació, puc revocar el consentiment que ara presto. En la meva presència s'ha donat al/la pacient tota la informació pertinent, adaptada al seu nivell d'enteniment i està d'acord en sotmetre's a aquest tractament/procediment. Per això, manifesto que em considero satisfet/a amb la informació rebuda i que comprenc la indicació i els riscs **més freqüents** que poden aparèixer a nivell generals durant la realització d'aquest **procediment quirúrgic i/o terapèutic** així com els riscos concrets que poden aparèixer en el cas del/la pacient donada la seva situació clínica i les meves circumstàncies personals (riscs personalitzats) que són:

_____.

I en tals condicions dono el meu consentiment informat per que es practiqui el següent procediment:

Excisió de gangli limfàtic axil·lar. Buidament ganglionar axil·lar. Mastectomía / Tumorectomía.

Girona, _ _ / _ _ / _ _ _ _

Signatura del pacient	**Signatura del representant legal**	**Signatura del metge**
DNI:	**DNI:**	**Nº col·legiat:**

ANNEX 4

Hospital Universitari de Girona Doctor Josep Trueta

Generalitat de Catalunya
Departament de Salut

CONSENTIMENT INFORMAT PER A PROCEDIMENTS QUIRÚRGICS I/O TERAPÈUTICS

DESCRIPCIÓ DEL PROCÉS:

TRACTAMENT AMB QUIMIOTERÀPIA I/O TERÀPIES BIOLÒGIQUES
El tractament de la seva malaltia aconsella l'ús de quimioteràpia i/o teràpies biològiques. Aquests medicaments intenten eliminar les cèl·lules tumorals però no es pot evitar que actuïn també en les cèl·lules normals i amb freqüència produeixen efectes tòxics, que en general es poden prevenir o controlar amb les mesures adequades, però en alguns casos la toxicitat pot aparèixer. La via d'administració més comú és per la vena o en pastilles, però alguns tractaments poden requerir altres vies, com per exemple la espinal.

RISC GENERAL:

Qualsevol exploració, tractament o intervenció quirúrgica presenta riscs generals. El més greu és la possibilitat de parada cardíaca. Altres complicacions són les hemorràgies i infeccions. En cas d'urgència vital, caldrà actuar sobre aquestes complicacions amb els mitjans oportuns per al bé del pacient, dels que s'informarà (sempre que les circumstàncies ho permetin) el malalt o la persona que en sigui responsable.

RISCS ESPECÍFICS:

La toxicitat més freqüent que pot aparèixer amb el tractament inclou en major o menor grau:
Reaccions al·lèrgiques al medicament. **Nàusees i vòmits. Diarrees. Ulceracions** a la boca i al tub digestiu. **Caiguda** del cabell i pèl corporal. **Alteracions** de la pell. **Alteracions** de la sensibilitat: formigueig i sensació d'adormiment a les extremitats. Disminució de l'audició. **Extravasació**; sortida del medicament fins a l'exterior de la vena, irritant la pell o produint lesions més severes. **Disminució** de les cèl·lules de la sang; de **glòbuls vermells** (cansament o dificultat per respirar); de **glòbuls blancs** (augment del risc d'infeccions); de **plaquetes** (augment del risc de sagnat). En **menor freqüència** es poden produir alteracions tardanes en òrgans (pulmons, cor, ronyons, medul·la òssia). També pot augmentar la possibilitat d'aparició de segons tumors.

AUTORITZACIÓ

Signatura del pacient

DNI:

Signatura del metge

Nº col·legiat: