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Tesi di dottorato

Cyanoacrylate based glue in reducing seroma after axillary dissection. Preliminary results of GLUBREAST Trial.

IL RELATORE

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LA CANDIDATA

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Dedicata a mio padre,

che vive oggi grazie alla Ricerca,

che tante volte si è sentito un numero senza volto nei trials randomizzati a cui ha preso parte, una cartella clinica nei comitati multidisciplinari in cui è stato discusso il suo caso.

Essere uno di quei numeri gli ha permesso di andare avanti, di continuare a combattere.

E' per questo che guardando lui penso ogni giorno che la Ricerca è Vita…

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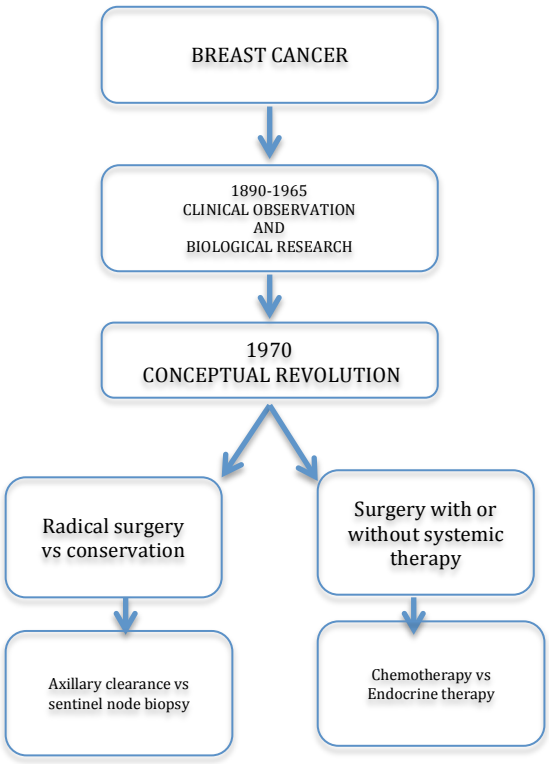
1.Introduction

1.1 Randomised trials in surgery

The stereotype of surgeons as technologists who are not truly dedicated or almost indifferent to research persists in the collective imagination of the scientific world[1]. The role of the surgeon today, however, is rapidly evolving and is being transformed in many cases into what the Anglo-Saxons call "Surgeon-Scientist". In this new version of himself the surgeon tries to recognize the limitation of knowledge, reject unconditional "compliance" towards dogmas and absolutisms and tries to take part in clinical research, contributing significantly with the realization of clinical studies and the implementation of scientific resources available. The nature of "surgical" research is mainly carried out in testing new techniques and innovative technologies aimed at the development of minimally invasive procedures that improve patients' expectations and quality of life. Moreover, an important advance in surgical research has been the development of psychometric tools for the adequate assessment of the quality of life[2].

Breast cancer represents a typical model of research applied to surgery (Fig.1). The high incidence of breast cancer, the "long-survivorship" of women who suffer from it, the high cosmetic expectations and the impact on the quality of life perceived by patients are its trigger and fuel.

Fig.1 Surgical research and breast cancer



Randomised controlled trials (RCT) are experimental studies aimed to evaluate the effectiveness of a specific treatment in a certain population. To be defined as such, a study must have the following characteristics:

1. **Experimental (trial):** the procedures for assigning subjects to the population to be studied are established by the experimenter. Once the population has been recruited, on the basis of all the variables of known prognostic significance considered by the researcher, the effect of one treatment occurs comparing it with the effect of another different treatment.

2. **Controlled (controlled**): the subjects involved in the study are divided into two groups: the experimental group or arm that receives the treatment, and the control group or arm that receives a different or no treatment. If the experimentation is carried out correctly, the two groups are as homogeneous as possible, at least for all the variables considered, and therefore comparable.

3. **Randomised (randomised):** the assignment of the treatment to the subjects must be done with a random method (random). Randomization increases the probability that other variables, not considered in the study design, are distributed evenly in the experimental and control groups. In this way, any differences observed between the two groups can be attributed to the treatment.

Why are randomised trials so important?

When the natural history of a disease like cancer is unpredictable, random errors or systematic errors are more than likely to confuse the results of a study. The effect of surgery should therefore be assessed through randomised controlled trials. The results of a randomised controlled trial, if the trial is designed correctly, will allow the intelligent clinician to evaluate the benefits against harm and thus to provide the patient with sufficient knowledge to make rational decisions based on their values, fears and expectations.

RCT and evidence-based medicine (EBM) are presently in the forefront of the physician's thinking in the decision-making process for therapeutic intervention. Evidence-based medicine is a clinical method designed for the transfer of knowledge from scientific research to the care of individual patients. David L. Sackett,

considered the father of the method, called it "the explicit and conscientious use of the best scientific evidence in making decisions in medical practice". To facilitate the identification of the best evidence by clinicians, EBM has defined a hierarchy of sources of knowledge in medicine.

According to the "pyramid of evidence", the most reliable sources for EBM are the systematic reviews followed by randomised controlled trials. However, there are some critical issues and some limits to the implementation of randomised controlled trials, especially in the surgical field. First, randomised studies of technically demanding procedures cannot often be conducted due to the different skills and abilities among surgeons in the scientific community. However, this mix of skills is precisely why randomised controlled testing is important - for assessing the generalizability of the technique and for the indirect effect of auditing surgical outcomes. In a large pragmatic study the entire spectrum of surgical skills would be represented and, provided that each surgeon has his own randomization set, there should be no prejudice in the outcome of the total sample and the result achieved would be generalizable across the community. At the same time, if there is a data monitoring and security committee that reviews the results of intermediate analyses, surgeons with unacceptably high morbidity or mortality can be readily identified and retrained. The final result of this study would therefore be to also evaluate the applicability of a technique among a group of surgeons with sufficient skills.

If the operation were so technically demanding that only a handful of surgeons could perform it competently, it would not be worth divulging that procedure, except

perhaps for procedures for extremely rare conditions to be treated in centres of excellence. Secondly, surgical experiments are frequently non-profit and require investments of energy and human resources that are poorly paid or even unpaid, who prefer to work hard to complete the routine rather than dive into the extra-ordinary only for the sake of research, a feeling not common to all . The training and training course of a surgeon's life tend to favor manual activities, practical activities, doing rather than thinking. The anxiety to learn, to laboriously overcome the learning curve of one or more standard surgical techniques, often remove young surgeons from research, especially if involved in non-elective but emergency surgery[3].

1.2 The impact of randomised trials in breast surgery

The surgical history of breast cancer could exemplify how research has influenced its treatment. William Halsted demonstrated the safety and efficacy of radical mastectomy in the treatment of early breast cancer about 130 years ago. However, efficacy in this context concerns local disease control. But what was the impact of Halsted's mastectomy on a woman's quality of life? Over the past 30 years there has been a paradigm shift in the understanding and management of breast cancer[4]. Oncologists, following Bernard Fisher's studies[5] now consider the disease as a systemic disorder in most cases at the time of clinical presentation. There have been two important therapeutic consequences of this paradigm shift: conservative breast surgery and adjuvant systemic therapy. Breast conservation techniques consisting of

surgery combined with radiation therapy have produced survival and local relapse rates equivalent to those of radical surgery after nearly 30 years of follow-up[5,6]. The research on conservative surgery techniques has been risen by the intuitive belief that women who keep their breasts report a better quality of life than those who have undergone mastectomy, measured by the degree of anxiety, depression and sexual adaptation. This area of study is a good example of how quality of life assessments not only revealed counter-intuitive results, but also generated second-order hypotheses that allowed surgeons working alongside psychologists and clinical nurses to improve subjective results for all patients regardless of primary breast cancer management[7]. Another important step in the development of surgery for women with breast cancer was the publication of the world's first overview of studies on systemic adjuvant therapy[8]. The result has been a significant reduction since 1985 in breast cancer mortality. Furthermore in the 1990s, the sentinel node biopsy study of the UK Medical Research Council (MRC) was born which showed how much a randomised controlled trial designed for an emerging technology in the management of early breast cancer could change in such a way determining axillary surgery in selected cases. Historically, complete dissection of the three levels of axillary lymph nodes was considered essential in the management of early breast cancer, not only for local control of the disease but for its possible cure. The MRC study, together with the Italian study by Veronesi et al[10] and the American study by Krag et al[11], showed that overall survival in patients with early breast cancer is not affected by complete dissection of the axillary lymph nodes, but this procedure remains important for local

disease control and patient selection for adjuvant systemic therapy. In addition, patients present earlier in the natural history of the disease, so that up to 70% of patients who have undergone lymph node surgical clearance, there is no histological evidence of invasion[12]. These patients therefore underwent a technically complex procedure, with relative morbidity, due to the modest advantage of learning that they have a good prognosis. To date, there is no reliable imaging technique for preoperatively indicating whether lymph nodes are affected or not. For some time it has been recognized that in most cases lymphatic drainage from the breast passes through one or two "sentinel" lymph nodes before going distally to the first, second and third anatomical division in the axilla. If these sentinel nodes are clear, then "skip the lesions" in the upper armpit levels are extremely rare. The subdermal injection of a radioactive labelled colloid material with or without a blue dye can reveal this sentinel node at a very early stage of the surgical procedure, or even as an outpatient procedure before final surgery. The assumption is that if the node it is histologically negative, further axillary dissection is not necessary, but if the node is affected, surgical authorization or radiation therapy may be appropriate. Unfortunately, the procedure requires a nuclear medicine department with expensive equipment. Furthermore, there is not only discussion about the prevalence of lesions to jump higher in the armpit, but also about the recognition that the real sentinel node can be completely erased by cancer, with the lymph diverted on a higher group of nodes. Finally, the techniques are not simple. The MRC trial allows an initial phase during which surgeons develop the necessary skills. In the second phase, patients will be

randomly assigned to management based on the status of the sentinel node only or the status of the axillary contents as conventionally defined. Outcome measures will include short-term physical and psychological morbidity and long-term local control and survival. The differences in survival will not concern the removal of the lymph nodes per se, but the adjuvant systemic therapy chosen based on the estimate of the involvement of the axillary node. Regardless of the treatments being compared, patients do better in a controlled study than out of it, so in addition to advancing knowledge for the benefit of future generations, participation in a randomised controlled trial is the best means of limiting risk for today's patients.

1.3 Randomised controlled trials and medical devices

Medical devices (MD) play an important role in the diagnosis, prevention and treatment of diseases. However, with respect to pharmaceutical products, there is no strict formal regulation for the demonstration of benefits and exclusion of harm to patients[12]. The growing number of scandals followed by the withdrawal of medical devices from the market recently led to the proposal for a regulation of the European Parliament and of the Council relating to medical devices issued on 26 September 2012. However, the proposal has been intensely criticized. The panel of experts of the European Clinical Research Infrastructure Network (ECRIN) requested, in fact, the application of a more rigorous clinical evaluation of medical devices: "[...] high and medium risk devices (active and inactive implantable medical devices (classes III and

IIb) as well as in vitro diagnostic devices need a crucial clinical evaluation before market approval "[13,14].

The team focused on increasing patient safety with adequate scientific evaluation of the benefits and harms, both short and long term, based on the results of randomised controlled trials (RCTs) and other clinical trials. The pre-marketing assessment and approval of the high and medium risk medical device should be associated with on-going post-marketing surveillance to ensure that the benefits and harm of applying the device in the real world are similar to existing clinical trial data". The medical device industry argues that the hierarchy of classical tests cannot be applied to medical devices, as it is impossible to carry out randomised clinical trials[12].

Systematic literature searches without meta-analysis and internal communications to the European network of clinical research infrastructures (ECRIN) conducted from 2013 to 2017 as part of the ECRIN Integrating Activity (ECRIN-IA) project in addition to the existing barriers for all studies, have identified three main obstacles for randomised clinical trials on medical devices, namely:

(1) Randomization, including assessment times, acceptability, blinding, comparison group selection and learning curve considerations;

(2) Difficulty in determining the appropriate results;

(3) Lack of scientific advice and specific regulation.

With respect to evaluation times, it is necessary to admit that choosing the most appropriate time to evaluate a medical device clinically is a specific problem to consider. Medical devices can often undergo frequent changes in the design of the device after their first introduction into humans. Older products will become obsolete when a new device proves more effective and / or safer. The medical device industry is concerned that a clinical trial, which in the best case takes 2-3 years to complete, will get results at a time when the new device is already out of date.

Secondly, a particular feature of the healthcare technologies that use MDs is that surgeon's experience and expertise often influence the results of the technique. Surgeon's experience and expertise can lead to differences in performing the operations. A lack of experience can influence the outcome of the study, penalizing the new treatment tested. Many reports indicate that the operations performed during healthcare providers are in a learning phase that is associated with greater risks and adverse events than the operations performed after the workout has been completed. The learning curve for surgeons should be taken into consideration when evaluating surgical or interventional techniques. Defining results from clinical trials on MDs as relevant is complex. This is partly due to the great application variability for different types of medical devices such as pacemakers, insulin pumps, operating room monitors, defibrillators and surgical instruments, and partly due to a large variety of potential relevant outcomes. In a multi-stakeholder seminar of the Joint European Forum for Good Clinical Practice and the MedTech Working Group on Medical Technology in Europe Luxembourg, in October 2015, regulatory barriers to the

development of medical device studies were identified and discussed recommendations for improvements[14,15].

2. GLUBREAST Trial

2.1 Background

Breast cancer represents the most frequently diagnosed cancer in women. One in eight women receive a diagnosis of breast cancer in the Western world during her life. Around 52,000 women have been diagnosed a breast cancer in 2019 in Italy. Nowadays, 70% of women diagnosed with breast cancer is scheduled for breast conserving surgery and sentinel node biopsy. The remaining one third of women with breast cancer still undergo axillary lymph node dissection as they present with clinically palpable and / or ultrasound detected axillary lymphadenopathy.

Axillary lymph node dissection is characterized by the risk of complications that tend to occur in 15 - 81% of cases according to existing literature[15]. Such complications include seroma, haematoma, paresthesia, lymphocele, lymphorrhea and lymphedema. The most frequently reported complication in the literature is seroma, as a result of the bloody tissue that activates fibrinolysis in serum and lymph with subsequent collection of exudate in the spaces left free by the surgery itself [16-18]. The post-operative management of the seroma is regulated by the use of drainages, whose prolonged stay in the armpit tends to significantly increase the risk of infection of the surgical site and consequently to delay the start of adjuvant therapies[19-20].

The onset of an axillary seroma requires several outpatient visits in order to check the status of the drainage or to manually aspirate the collections with a fine needle, causing an increase of costs in terms of materials and workforce and at the same time a moment of psychological distress for patients.

Numerous devices and techniques have been used to obliterate the axillary cavity created by surgery, through transposition flaps, sclerosing substances, fibrin glues, sealants and drugs such as octreotide, devices that have often shown discordant and scarcely significant results[20-22].

2.2 Rationale

The decrease of post-surgical seroma can reduce the number of post-operative outpatient visits and the consequent risk of infection associated with long-term drainage or numerous evacuative fine needle aspirations. Cyanoacrylate based glue sponsored and advertised as noteworthy MD able to reduce axillary seroma after breast surgery. One single-blind study has shown encouraging results in reducing seroma after pelvic lymphadenectomies in 2014[23].

Cyanoacrylate based surgical glue is a biodegradable synthetic cyanoacrylate adhesive, modified by adding a monomer; is a class III medical device certified CE 0373 (Italian Higher Institute of Health) for internal and external surgical use. In that class, all medical devices have the highest risk possible, and permanent monitoring is required during their lifetime.

Cyanoacrylate based surgical glue has shown haemostatic and adhesive properties in surgical sites others than axilla and once solidified it creates an antiseptic barrier against the most common infectious or pathogenic agents in surgical operations[23-30].

Cyanoacrylate based surgical glue in a humid environment, polymerizes quickly creating a thin and elastic film with high tensile strength that guarantees a solid adhesion to the fabrics. This film independently conforms to the anatomy of the tissues, is waterproof and is not affected by blood and organic liquids[24-46].

In 2017 one single institution study on 128 women has shown that cyanoacrylate based surgical glue can contribute to the reduction of seroma after mastectomy and consequently reduce the permanence of post-operative drainages or the frequency of manual aspirations[47]. On the other hand in the same year the Australian study from Clement et al on 76 women did not show any benefit in the use of cyanoacrylate based glue in axillary surgery in reducing the risk of seroma formation[48].

It is therefore essential that other studies on larger samples can confirm or reverse these results.

In the light of the foregoing considerations, we set up a randomised controlled phase III trial, called GLUBREAST Trial aimed to verify the effectiveness and the safety of the aforementioned cyanoacrylate based surgical glue introduced by a nebulizer inside the axillary space after axillary clearance for breast cancer (Fig.3).

Fig.3 Nebulizer containing cyanoacrylate based surgical glue

2.3 Aim of the study

The aim of this study is to verify the efficacy of nebulized cyanoacrylate based surgical glue, namely (N-Butyl-2-CyanoAcrylate + Metacryloxisulfolane [NBCA + MS]), in the reduction of seroma after breast conserving surgery or mastectomy and ipsilateral axillary dissection in patients with breast cancer.

Primary Endpoints:

Evaluate the effectiveness of cyanoacrylate based surgical glue in reducing the total volume of serum drained after surgery

Secondary Endpoints:

- Evaluate the effectiveness of cyanoacrylate based surgical glue in reducing the total volume of serum aspirated manually after removal of the drainage
- Assess the possible extension of drainage beyond the 15th day
- Evaluate the number of manual aspirations of the seroma
- Evaluate cyanoacrylate based glue effectiveness within Body Mass Index categories
- Assess the incidence of adverse events in the two arms
- Evaluate the incidence of surgical site infection, haematoma, re-hospitalization, re-intervention

2.4 Variables to investigate

Primary endpoints measures:

Total volume of drained serum (ml) during the first 15 days

Secondary endpoints measures:

- Total serum volume after drainage removal between the 15th and 21st day
- Total number of needle aspiration between the 15th and 21st day
- Prolongation of drainage beyond the 15th day
- Body Mass Index (Kg/m^2)
- Surgical site infection, haematoma, re-hospitalization, re-intervention

3. Materials and Methods

3.1 Selection criteria

Inclusion criteria

- Age ≥ 18 years
- Female gender
- New diagnosis of breast cancer
- Breast conserving surgery or radical mastectomy along with axillary dissection
- Signed informed consent

Exclusion criteria

- Male gender
- Previous radiotherapy to chest-wall
- Neo-adjuvant chemotherapy
- Immediate breast reconstruction
- Uncontrolled psychiatric disease
- Lack of informed consent

3.2 Study design

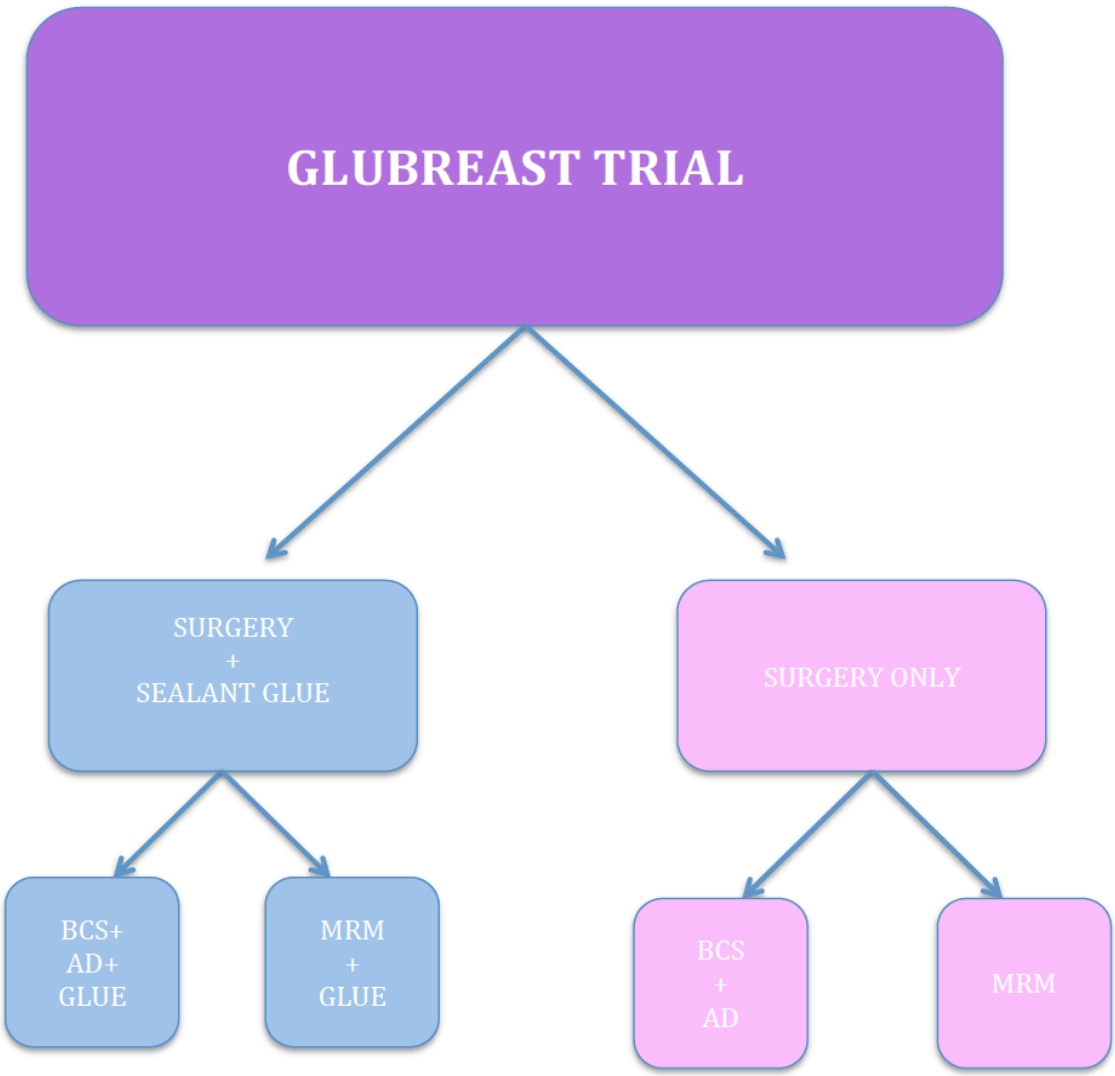
The study is a single centre randomised controlled phase III trial. The trial was set up within the Department of Breast Cancer at Nation Cancer Institute of Naples – IRCCS – "Fondazione G. Pascale", Naples, Italy. The Principal Investigator (PI) is a breast surgeon Raimondo di Giacomo, MD. Eleven more investigators, including me were involved within the same institution. The study was launched in May 2018 after Internal Ethical Committee (CEI) Approval on March 14th 2018 reference number12/18 and after resolution of April 10th 2018 number 448.

Study population is split as follows (Fig.2)

- **Experimental Arm:** Radical mastectomy or breast conserving surgery along with Axillary Dissection + cyanoacrylate based surgical glue application

- **Control Arm:** Radical mastectomy or breast conserving surgery along with Axillary Dissection without glue

Fig.2 GLUBREAST Trial. Study design



BCS Breast Conserving surgery

MRM Modified Radical Mastectomy

AD Axillary Dissection

3.3 Allocation and randomization

Eligible patients, after being illustrated the trial and have signed the informed consent, are randomised using a centralized randomization system by logging into the GLUBREAST Trial homepage at created by IBIS Information Systems: https://glubreast.ibisinfo.online/Public/homepage.php.

Each of the co-investigator can enter the program with personal username and password. The PI and the co-investigators can enter all the information on the demographic characteristics including Body Mass Index, type of surgery required, type of tumour inside the e-CFR, and the system assigns the subject to one of the two treatment arms according to a predefined randomization generated by the computer. If patients are allocated to Experimental Arm a green label is stuck on the patient discharge form, if to the Control Arm a red label is stuck on the patient discharge form. Computerized randomization creates population stratification. The type of surgery will constitute a layering level.

3.4 Sample size

The study population is composed of patients diagnosed with breast cancer who require breast conserving surgery or radical mastectomy with ipsilateral axillary dissection. The total sample size estimated is 200 patients. The sample size was calculated considering the total volume of drained serum (ml) during the first 15 days as the primary endpoint of the study. Assuming an incidence of seroma of 50% after axillary dissection our study will have power of 85% to show seroma incidence in Experimental group is 25%. Assuming an allocation ratio of N1:N2 and alpha level of significance was set at 5%, the sample size will be of 190 patients, with 95 subjects in each arm, because the primary endpoint in terms of effect size (which corresponds to an effect small-medium size) using a t test for the comparison of independent two-tailed samples. The final size was increased to 100 patients per group in order to limit the effect of any losses during follow-up.

3.5 Statistics

All statistical tests will be two-sided. Summary tables will illustrate the quantitative parameters such as mean, standard deviation, median, interval (minimum and maximum). The chi square test will be used for the statistical comparison between the obtained frequencies and the Student test for the continuous variables at the end of the study. Continuous variables without normal distribution will be analysed after applying an appropriate mathematical transformation or using Mann Whitney's nonparametric test. The normality of the distribution will be assessed according to the Shapiro-Wilks test. All registered and calculated variables will be presented in tables and lists using standard procedures depending on the type of variable below. The descriptive statistics on the ordinal and categorical variables will include numbers and percentages, while for the continuous variables the average, median, standard deviation and interval (minimum and maximum) will be reported. If relevant, the number of missing data will also be reported. Confidence intervals will be calculated at 95%. Demographic data (age, gender, body mass index, etc.) and other basic characteristics will be summarized and reported in tables and lists.

Primary efficacy analysis:

The total volume of serum in the two study groups will be analysed using Student's t test or using Mann-Whitney's nonparametric U test in case of evidence of non-

normality. Secondary efficacy analysis: The total volume of drained serum (ml) will be compared between the two groups by performing the Student t test for independent samples or the Mann-Whitney non-parametric U test in case of evidence of non-normality. The number of required evacuation punctures (n) and the control number of visits after discharge will be compared between the two groups using the Mann-Whitney nonparametric U test. In the analysis by subgroups the total volume of serum in the two study groups will be analysed using Student's t test or using Mann-Whitney's nonparametric U test in case of evidence of non-normality. The subgroups considered will be based on the surgical procedure performed: total or partial mastectomy and according to the categories of body mass index: normal weight, overweight, obese. The incidence of complications associated with the lymphadenectomy procedure, prolongation of drainage beyond the 15th day, infection of the surgical site, haematoma, re-hospitalization and re-intervention between the two groups will be analysed using the exact Fisher test.

Security analysis:

The descriptive statistics will be used to summarize the safety variables (e.g. all adverse events). Statistical analysis will be performed with SPSS software version 21.0 for Windows**.**

3.6 Follow up and study length

The follow-up period is three months. It consists in the evaluation of the quantity of serum found in the drainage from the first post-operative day up to the fifteenth post-operative day by investigators during outpatient clinic. Then, from the twenty-first day up to a maximum of 90 days, the number of needle aspirations (if necessary) and the volume of the fluid are assessed. During the follow up possible side effects are also monitored. The PI and all other co-investigators are asked to fill a discharge form reporting drained volumes, number of aspirations needed after the 15^{th} day, and complications or side effects such as surgical site infection, haematoma, fever, allergic reaction, or others than listed (Fig.4). The total duration of the study is 48 months from May 2018 until the conclusion of the follow-up and statistical analysis of the data.

Fig. 4 GLUBREAST Trial discharge and follow up form

Number of Randomization: ***Label Stick***

	Discharge date		7th post-op day		15th post-op day		21th- post op day		Days after 21th	
Drain (ml)										
Fever	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
Infection	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
Allergies	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
Haematoma	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO
Pain	YES	NO	YES	NO	YES	NO	YES	NO	YES	NO

Notes……..

3.7 Standard operating procedure and monitoring

The PI of the study (Raimondo di Giacomo, MD) selected eleven co-investigators, all breast surgeons with specific skills in clinical trials. They all obtained Good Clinical Practice (GCP) certificate before joining the trial. PI and all co-investigators are

allowed to recruit and randomize patients. All co-investigators are allowed to complete trial discharge form and to see patients in the outpatient clinic. PI and two out of eleven co-investigators together with a data manager are allowed to complete e-CRF from the database for follow up reporting data collected by discharge forms.

All surgeons were trained to use cyanoacrylate based surgical glue by product specialist in the operating room. When a patient is assigned to Control Arm standard breast conserving surgery or mastectomy along with axillary dissection in performed by one of the investigators. At the end of surgery a suction drainage of 19 French calibre is put in the axillary cavity and fixed with a silk stitch. When a patient is assigned to Experimental Arm, after breast surgery either conserving or radical mastectomy along with axillary dissection is performed, the investigators load 1 ml of glue on a small syringe. The syringe is screwed to a nebulizer and after opening the safety valve but switching it on the "ON" button the nebulized glue is applied in the empty axillary space at two centimetres distance for about 60 seconds (Fig. 5, 6, 7). Then the glue dries very fast and once it is crystalized a suction drainage of 19 French calibre is put in the axillary cavity and fixed with a silk stitch. At discharge the fluid collected in the drainage is reported in the form and the drainage is emptied out. Patients go home with drains and come for outpatient visits at day 7^{th}, 15^{th} and 21th. At day 7^{th} and 15^{th} the drains are emptied out by investigators and fluid is measured and reported in the form. After day 15^{th} drain is removed and if seroma is still in the cavity it is aspirated by fine needle puncture.

Trialists have been monitoring by auditors coming from the Scientific Committee from the National Cancer Institute. To date two "audit" by Internal Scientific Advisory Board have been assessed. One on the 6th May 2019, the other on 15th July 2019 and the final report has been uploaded on the SMART platform of National Cancer institute of Naples, which represents the intranet scientific platform used by the Advisory Board and the Internal Ethical Committee (CEI) to update and control on-going clinical and preclinical trials.

Fig.5 One of the investigators loads the syringe with 1 ml of cyanoacrylate glue.

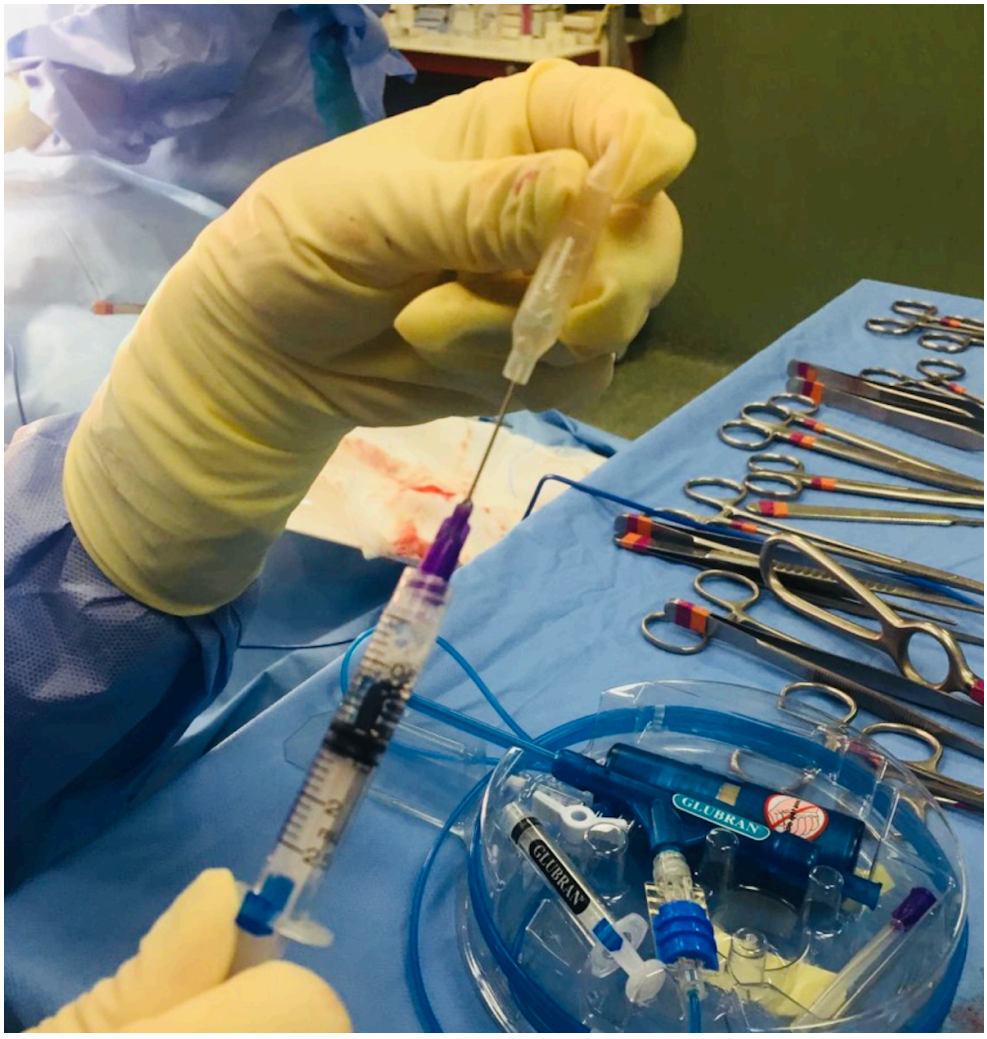


Fig.6 Application of cyanoacrylate glue in the axillary cavity after axillary dissection (Experimental Arm - GLUBREAST Trial)

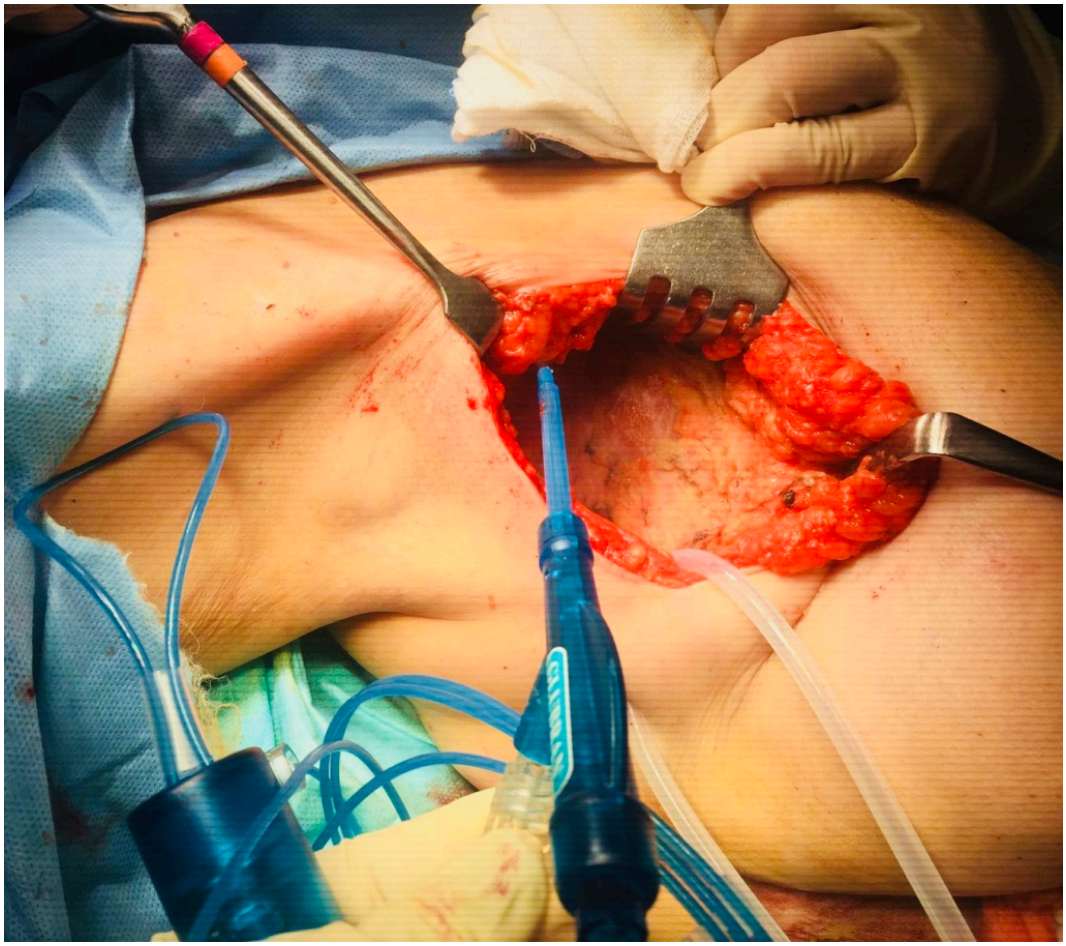
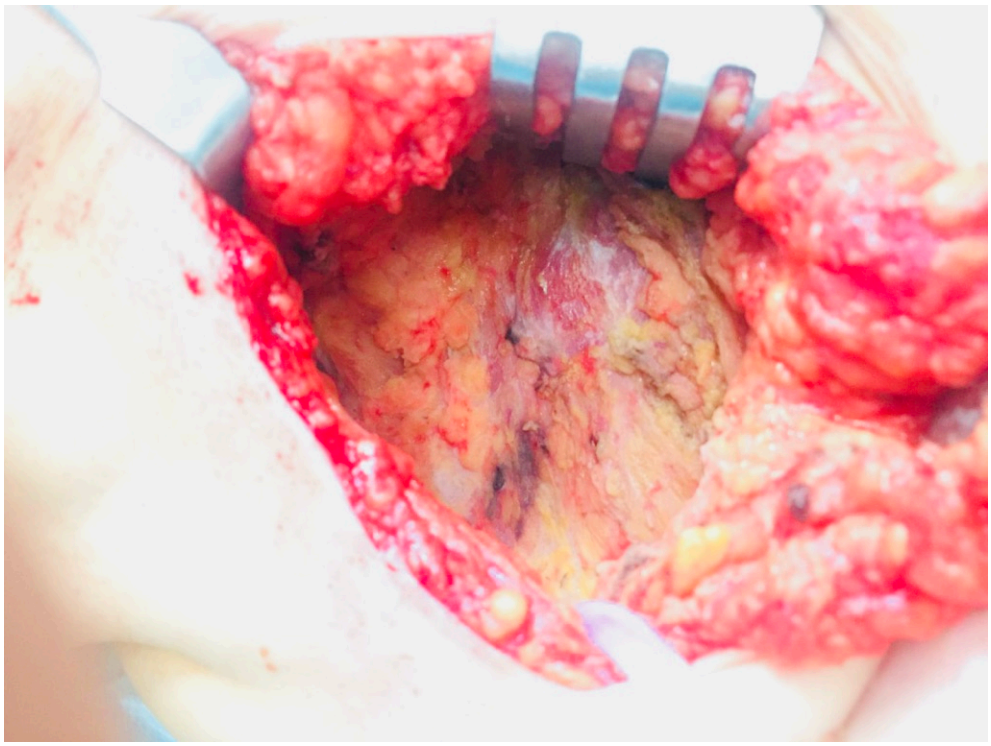


Fig.7 Axillary cavity after axillary dissection and application of cyanoacrylate based glue. Anatomic structures are "crystalized" by the glue (Experimental Arm – GLUBREAST Trial)



4. Results

88 patients were randomised in the GLUBREAST Trial from May 2018 to December 2019. The trial is still recruiting and will stop when the pre-specified sample size (200 patients) will be reached. Only preliminary results are here shown in table 1.

Results were extrapolated by discharge form validated by the Internal Ethics Committee and periodically checked during the insight visit of the Scientific Advisory Board monitors. Data from all 88 patients have been analysed. 43 patients are in the Experimental Arm and 45 are in the Control Arm. Mean age is 55.5 years. Mean Body Mass Index is 29.51 Kg/m^2 (Standard Deviation 6.51) in the Control Arm and 29.46 Kg/m^2 (Standard Deviation 5.46) in the Experimental Arm.

Regarding the primary endpoint, the total volume of serum drained in the Experimental Arm is higher than the Control Arm (951.15 ml ± 427.0 ml Standard Deviation vs 795.3 ml ± Standard Deviation 560.9 ml), $p < 0.004$.

When looking at secondary endpoint in terms of safety we can conclude that till now none of the patients enrolled in the Experimental Arm of the study and therefore treated with cyanoacrylate based glue reported adverse reactions, such as allergies, redness or fever. None of the patient reported itching, eczema or pain. Two of the patients from the Experimental Arm were re-hospitalized and re-operated because of positive margins after breast conserving surgery. One had margins cavity shave, the other had mastectomy. Neither re-operation nor re-hospitalization was due to adverse reaction to the glue. Only one in 43 patients had a haematoma (2.32%) in the Experimental Arm, whereas four out 45 patients had haematoma in the Control Arm (8.88%). One surgical site infection was reported in the Control Arm (2.22%), whereas none infections were reported in the Experimental Arm (0%). The prolongation of drains after the 15^{th} was found in 31% of patient. It was of eight days more in the Control Arm compared to five days more in the Experimental Arm (23

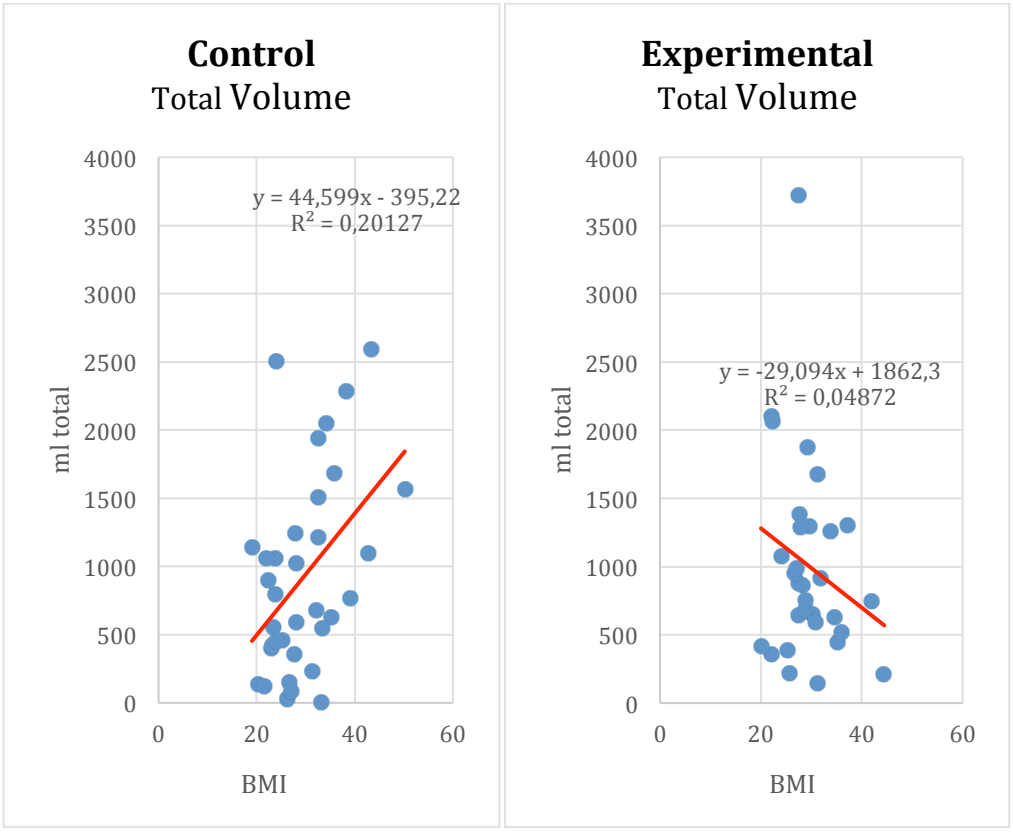
days vs 20 days). The mean number of fine needle aspiration after drain removal at day 15th was 1.06 (Standard Deviation 1.45) in the Control Arm and 1.10 (Standard Deviation 1.37) in the Experimental Arm. The total volume of serum aspirated manually after removal of the drainage at 21st post-operative day was 17.7 in the Control Arm (Standard Deviation 47.0) vs 14.8 in the Experimental Arm (Standard Deviation 28.0).

Table 1. Preliminary results of the GLUBREAST Trial

	Mean BMI Kg/m^2		**Total Volume (ml)**		**N° of aspirations after drain removal**		**Volume after drain removal**		**Prolongation of drain after 15th in one third**	**Surgical Site infection**	**Haematoma**
Experimental Arm	29.46	SD 5.46	951.15	SD 427.0	1.10	SD 1.37	14.8	SD 28.0	5 more days	0%	2.32%
Control Arm	29.51	SD 6.51	795.3	SD 560.9	1.06	SD 1.45	17.7	SD 47.0	8 more days	2.22%	8.88%

Interestingly among secondary outcomes we noticed that by stratifying patients according to BMI the production of fluid was directly proportional to BMI, whereas in the Experimental Arm, the more the BMI increased the more the fluid decreased. Figure 8 shows a scattered plot comparing total volume of drained fluid in the two Arms stratified by BMI.

Fig. 8 Total Volume drained stratified by Body Mass Index. Control Arm vs Experimental Arm



Analysing the production of fluid volumes by week we noticed that within the first two weeks after surgery the fluid is massively produced in the Experimental Arm and then tend to decrease very rapidly after the 15th day (third and forth week), whilst within the Control Arm the production of fluid is more constant and does not vary significantly by week (Fig.9, 10).

Fig 9. Drained volume by Body Mass Index per week (1st and 2nd week)

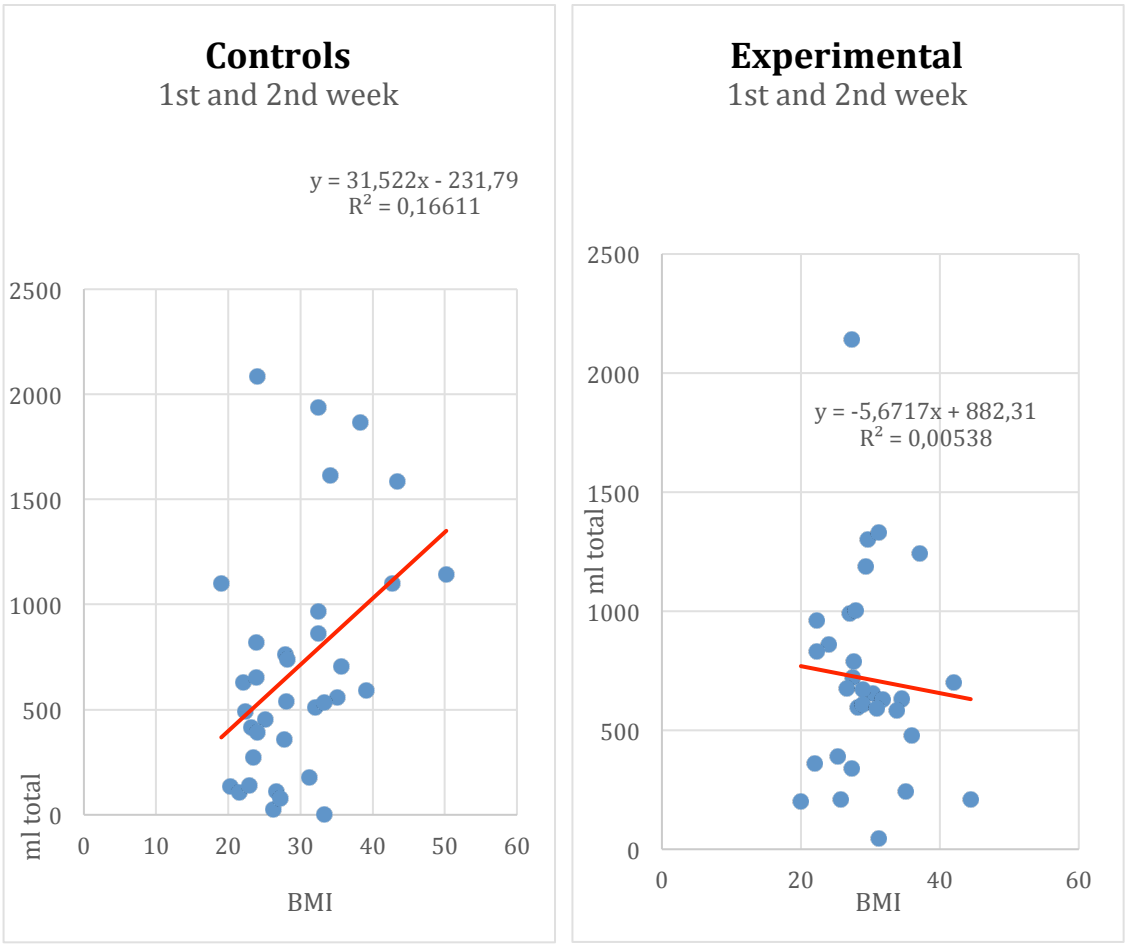
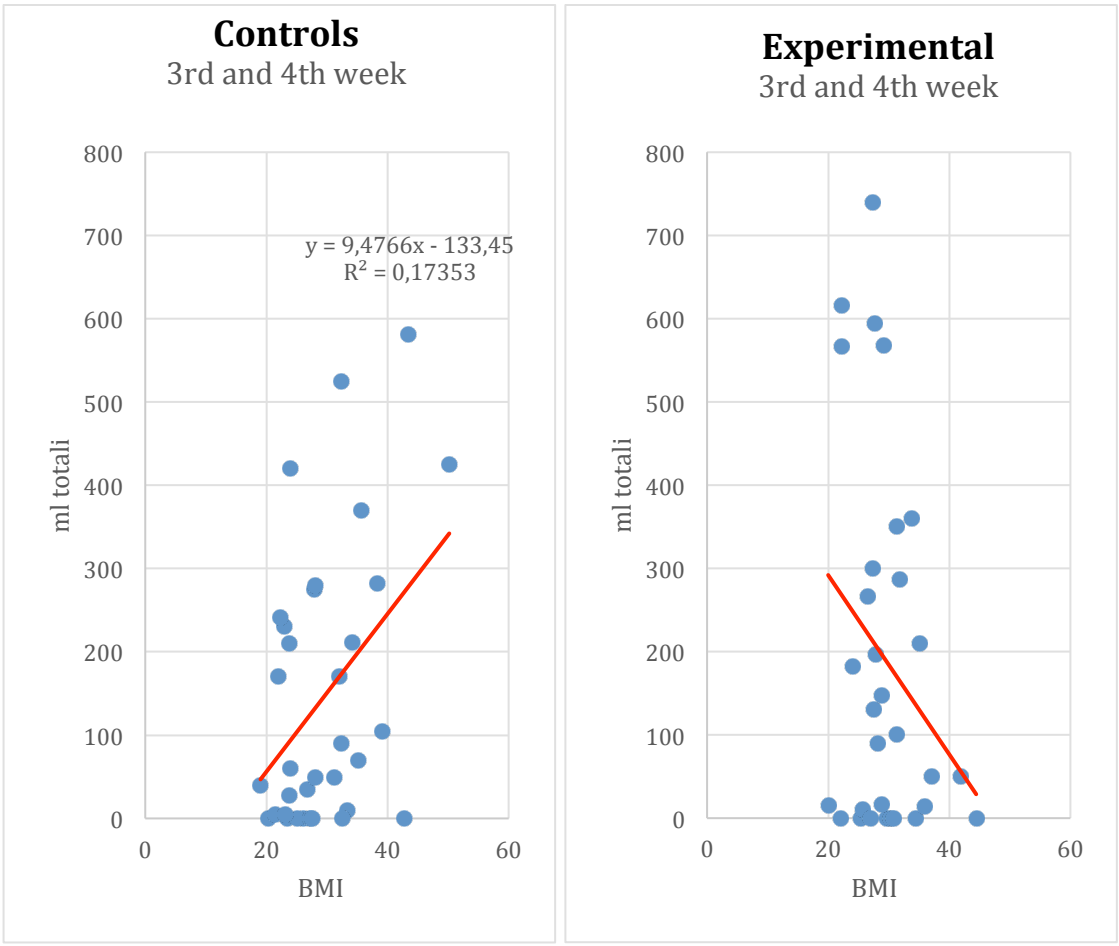


Fig 10. Drained volume by Body Mass Index per week (3rd and 4th week)



5.Discussion

Clinical studies such as randomised controlled trials are designed to answer specific questions, e.g. whether one drug is more effective than another at preventing a particular event occurring. What is the difference between primary and secondary endpoint? A primary endpoint is the specific event that the study is designed to assess the effect of one drug upon another. Secondary endpoints are additional events of interest, but which the study is not specifically powered to assess. Can we separate the wheat from the chaff? Opinions are sharply divided about secondary endpoints. Many believe that they are largely worthless ("hypothesis-generating only"). Meanwhile, others are willing to use them to guide management. Secondary endpoints aren't for hypothesis generation only. If we believe that secondary endpoints are good only for hypothesis generation we might stop and make some considerations. This belief can be disproven as follows. *Safety endpoints* are secondary endpoints. If a study shows as a secondary endpoint that a given intervention causes harm, that is something that should be taken seriously (often affecting management). Thus, the concept that secondary endpoints should never affect management is invalid. The ugly truth is that data quality isn't an all-or-none phenomenon. Instead, there exists a continuous gradation between low-quality and high-quality evidence. The primary endpoint of a multicentre RCT is the highest quality, but this is a rare treat in critical care. In practice, we are often forced to guide our clinical practice with evidence of intermediate quality. Cyanoacrylate based surgical glue is widely used

in surgery. It has shown encouraging results in thoracic surgery, colorectal surgery, upper gastro-intestinal surgery and in liver surgery acting like haemostatic and sealant by reducing re-operation rates, anastomotic leak rate and post-operative bleeding. Some groups have being used glue to reduce seroma and lymphorrea after lymph node dissection but scarce and discordant results have been published. Nevertheless cyanoacrylate based surgical glue products are advertised as medical devices able to reduce seroma in axillary and breast surgery, so most of the surgeons continue to use them without clear evidence and/or specific indications. To date GLUBREAST Trial represents one of the few trials testing medical devices. This is the case of a study where secondary endpoint have overcome primary. Actually, primary endpoint has not been reached. First of all because the total volume of drained fluid is higher in the Experimental Arm and secondly because in the statistics paragraph we pre-specified a 25 % reduction in axillary seroma incidence which was not respected. GLUBREAST preliminary results seem to be in line with the Australian study by Clement et al [48] showing no benefit in reducing axillary seroma when using cyanoacrylate based glue in breast surgery. Preliminary results from GLUBREAST Trial totally disagree with the Greek study by Vasileiadou[47] et al. However, secondary endpoints confirmed how safe the device is. Nor adverse event neither allergy were reported. Secondary outcomes shoes the device has haemostatic and anti-bacterial properties. With great probability at the end of the GLUBREAST Trial, investigators will conclude that cyanoacrylate based glue might be effective amongst obese women whereas

have no effect amongst normal weight patients. We might think that cyanoacrylate based glue roles a specific play on the fat tissue and adipocytes pathway responsible for seroma formation, but is not able to close lymphatic vessels of the axilla and to stop the production of proteins and electrolytic components of seroma. This is just a hypothesis to explain our results after stratifying patient by BMI. This may be the starting point for a newer study.

6.Conclusions

In conclusion, after preliminary data analysis GLUBREAST Trial is not showing clinical benefit with the use of cyanoacrylate based surgical glue in reducing the risk of seroma formation in the setting of breast surgery after axillary dissection. Experimental Arm and Control Arm showed no statistical significant difference in the total volume of fluid collected during follow up, disappointing the primary endpoint by now. On the other hand, secondary endpoint measures confirmed device safety and antibacterial and haemostatic properties. A new question rose up after primary analysis and this is whether cyanoacrylate based glue is effective amongst obese patients whom might be the target of this specific device in the future. Further preclinical and clinical studies are warranted to verify this interesting hypothesis. This is all we have for now, waiting for final results.

7.References

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