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Influence Of Neoadjuvant Chemotherapy Regimen On Breast Reconstruction Outcomes

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Influence of Neoadjuvant Chemotherapy Regimen on Breast Reconstruction Outcomes

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THESIS ABSTRACT

Title: INFLUENCE OF NEOADJUVANT CHEMOTHERAPY REGIIMEN ON BREAST RECONSTRUCTION OUTCOMES.

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Background: Neoadjuvant chemotherapy (NACT) provides a survival advantage in breast cancer. Prior to mastectomy, NACT shrinks tumor size and improves pathological response in breast cancer. Evidence to date has evaluated the impact of chemotherapy on autologous breast reconstruction outcomes as a binary variable. In this study, we evaluate the effect of specific NACT regimens and dosage on rate of post-operative complications after autologous breast reconstruction. We also evaluate the effect of NACT on circulating immune cells that orchestrates wound healing.

Methods: Following IRB approval, patients who underwent NACT and microvascular breast reconstruction at Yale New Haven Hospital between 2013 and 2018 were identified. Demographic variables, oncologic history, chemotherapy regimens, and complication profiles were abstracted from the electronic medical record. Chemotherapy regimens were stratified by inclusion of anthracycline and order of taxane. Statistical analysis identified significantly varying factors between complication and no-

complication cohorts. Chi-squared tests, Fischer exact tests, and t-tests were used for univariate analysis. Multivariate binary logistic regression was used to control for confounding variables.

Results: 100 patients met inclusion criteria. In a multivariate regression model controlling for significant covariates like BMI and ASA, the administration of taxane-first in an anthracycline-containing chemotherapy regimen was associated with increased complications (OR = 3.521, $p = 0.012$). In particular the administration of taxane-first was associated with a 2.5-fold increase in incidence of fat necrosis (OR = 2.481, $p = 0.040$). Logistic regression model evaluating the effect of taxane-first regimen on complication rates, the AUC was estimated to be 0.760 ($p < 0.0001$), particularly fat necrosis 0.635 ($p < 0.05$). There was no correlation between anthracycline inclusion and postoperative outcomes. The dosage of chemotherapy, the number of days between NACT completion and surgery, and the number of circulating immune cells did not have a significant effect on postoperative outcomes.

Conclusion: The administration of taxane first in an anthracycline-containing NACT regimen contributes to an increase in minor postoperative autologous breast reconstruction complications. particularly fat necrosis. Our report has the potential to inform the sequence of NACT administration but the benefits of taxane-first regimens in improving tumor outcome may outweigh increased post-reconstruction complication risk.

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Published work[1]

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INTRODUCTION TO THESIS

Breast cancer, excluding non-melanoma skin cancer, remains the leading cancer diagnosis in females around the world. In 2019, there was an estimated 268, 600 diagnoses in the United States, representing 30% of new cancer cases in women. Of this, 41,760 cases accounted for breast cancer deaths in the United States[2]. Despite advances in several non-surgical treatment approaches such as targeted immune and hormone therapies, chemotherapy and radiation; nearly 40% of patients require mastectomy of the diseased and often contralateral breast[3]. Extirpative surgery in the absence of reconstruction can lead to altered body image that impacts on sexual and psychosocial well-being[4]. Breast reconstruction impacts positively on a patient's psychosocial well-being[5]. Autologous breast reconstruction, performed using either pedicled or microvascular approaches, uses patient's own tissue and offers the advantage of a more long-lasting, natural-feeling breast[6].

Neoadjuvant chemotherapy (NACT) offers the advantage of reducing tumor burden and improving pathologic response [7-10]. NACT potentially offers a predictive value of long-term survival based on treatment response[11]. Indications for NACT have expanded to include treatment of early-stage and locally advanced breast cancer, irrespective of tumor size[12]. Just as autologous breast reconstruction has evolved from pedicled to microvascular to perforator flaps, neoadjuvant therapy has evolved as evidence has linked efficacy to the specific NACT regimen[13].

Reconstructive outcomes, in theory, may be affected adversely by the cytotoxicity of preoperative chemotherapy[14-18]. Several studies have explored the postoperative effects of NACT on overall oncological outcomes and more specifically, on breast reconstruction [14-16,18-22]. There are conflicting data on the actual effects of NACT on post reconstructive outcomes. Some studies report that NACT is significantly associated with minor post-operative complications among patients receiving NACT compared to those who did not[15,20,21] while others report no similar major and minor postoperative complication in both group[14,16,18].

Previous studies are limited because they did not evaluate the effect of specific regimens, the sequence of chemotherapy administration, NACT doses, and time between the last dose of chemotherapy and breast reconstruction operation. Moreover none of these studies considered the amount of circulating immune cells that are critical to wound healing after mastectomy and breast reconstruction[19,23]. This thesis aims to close these gaps in literature.

BACKGROUND

Breast Cancer: Epidemiology, Risk factors and Pathophysiology

Breast cancer is the most commonly diagnosed cancer worldwide and continues to be the leading cause of cancer mortality in women. In the United States, there is a projected incidence of 281,550 cases in 2021[24]. 1 in 9 women will develop breast cancer in their lifetime with peak incidence among 60 – 69 year old age group[2]. Age and female sex are the most common risk factors. Others include early menarche, late menopause, nulliparity, late age at first full-term pregnancy, and having a personal prior and/or family history of breast cancer[25]. About 20% of breast cancers worldwide can be attributed to modifiable risk factors including obesity, physical inactivity and alcohol use[26]. Approximately, 5 – 10% of breast cancer cases are hereditary and are largely attributed to BRCA gene mutations. BRCA gene mutations are most commonly found in persons of Ashkenazi ancestry. The risk of developing breast cancer increases to 60 – 85% with presence of BRCA-1 and BRCA-2 mutations[27].

The pathology of breast cancer typically arises from hyperplasia of epithelial cells from the breast ducts or lobules and eventually progresses to invasive carcinoma. Less commonly, invasive breast carcinoma such as angiosarcoma, primary stromal sarcomas and phyllodes also arise from nonepithelial cells of the supporting stroma. The exact mechanism that initiates this hyperplasia is unknown, however, similar to other solid tumors. It has been proposed that the genetic mutations that lead to gain or loss of function of certain genes may be responsible. TP53, a tumor suppressor gene is the most

commonly mutated gene[28]. Its repression has been seen in 41% of tumors. Mutation of genes in the family of cyclin D1, a cell cycle regulator has been shown to sustain the proliferation of tumor cells. Other frequently mutated genes that have been reported in early breast cancers include PIK3CA (30%), MYC (20%), PTEN (16%), CCND1 (16%), ERBB2 (13%), FGFR1(11%, and GATA3 (10%)[28].

The most common subtype of breast carcinoma is ductal carcinoma in situ (DCIS) which represents about 85% of carcinoma in situ. DCIS usually presents as microcalcifications that are detectable on mammography and typically involve a focal small area in one breast. In contrast, lobular carcinoma in situ (LCIS) arises from the lobules of the breast and is typically multifocal and bilateral. LCIS does not form microcalcifications making mammographic detection challenging. Both DCIS and LCIS highly increase the risk for developing invasive carcinoma[25]. Invasive carcinoma is most commonly adenocarcinoma, 80% originates from infiltrating ductal type. Other histological variants include lobular carcinoma, rarely, medullary carcinoma which accounts for 6% of invasive cancer, papillary, tubular, and mucinous or colloid carcinoma. Inflammatory breast cancer is an aggressive, fatal cancer as it spreads rapidly to the lymph nodes[24].

Breast cancer can also be classified based on immunohistochemistry or presence of receptors on the surface, cytoplasm or nucleus of breast cancer cells. The most common nuclear hormone receptor is estrogen receptor (ER) and progesterone receptor (PR). HER2, is a receptor tyrosine-protein kinase surface receptor and member of the human epidermal growth factor receptor family. The over-expression and amplification

of HER2 has been shown to play a significant role in the development of breast cancer. Expression of HER2 is generally a risk factor for more aggressive cancer while estrogen and/progesterone positive breast cancer usually have a more favorable prognosis[29].

Breast cancer: Screening, Diagnosis and Management

There is an increased rate of breast cancer diagnosis in the recent decade, however, the survival rate has greatly improved most likely due to timely detection of the disease. Early detection of breast cancer is crucial to survival as the process from hyperplasia to invasive cancer typically occurs over 10 – 20 years. Mammography which detects microcalcifications is the most widely used screening tool for detection of breast cancer. The U.S. Preventative Services Force recommends biannual screening mammography for women aged 50 to 74 years[30]. Other screening tools include Magnetic Resonance Imaging (MRI), ultrasonography, and digital breast tomosynthesis. Particularly, breast MRI and digital tomosynthesis is recommended to women with dense breasts, those presenting with lobular carcinoma and women 30 years and above with high risk of developing breast cancer[31]. This includes women with known or suspected BRCA gene mutations, significant family history, and previous exposure to mantle radiation to the chest. While MRI is more sensitive for detection of breast cancer, its high cost and low specificity makes mammography a more universal tool for breast cancer screening. Ultrasonography is not recommended for surveillance of breast cancer. Currently, self-breast examination has not been shown to reduce breast cancer mortality or increase detection of cancer between recommended screening mammography[32]. A study evaluating the utility of using ultrasound in addition to screening mammography in

women with elevated risk for breast cancer showed that although ultrasound plus mammography increased the diagnostic yield (from 8 to 12 per 1000 women, 95% CI 1.1-7.2) there was also an increased rate of false positive results therefore, lowering the positive predictive value[33].

The management of breast cancer often involves a multidisciplinary approach including medical, radiation, and surgical oncology depending on the stage of the disease. Image-guided core biopsy is required for definite diagnosis of breast cancer. Axillary ultrasound is used to screen for spread to lymph nodes. Estrogen, progesterone and HER2 have become important therapeutic targets and biomarkers for breast cancer[34]. Therefore, assessment of the presence of biomarkers such as ER, PR and HER2 via immunohistochemistry is required for all patients with invasive breast cancer. In ER+ breast cancer, the blockade of estrogen to the estrogen receptor is targeted most commonly with tamoxifen which acts as an estrogen antagonist in breast cells but an estrogen agonist in other tissues like the uterus and the bone. Raloxifene is a selective estrogen receptor modulator that acts similarly to tamoxifen except it has the additional benefit of being an estrogen antagonist in the uterus making it a suitable therapy for post-menopausal women at increased risk for developing uterine cancer. Aromatase inhibitors that prevent production of estrogen are also used in some subset of patients. The use of aromatase inhibitors over a period of 5 years have been shown to reduce breast cancer mortality by 15% compared to tamoxifen only[35]. Ultimately, the patient's clinical factors including risk of relapse, bone health, preference, and tolerability of side effects should be used to determine the agent of choice. HER2+ breast cancer is treated with

monoclonal antibody, Transtuzumab. Transtuzumab induces an immune-mediated response that downregulates HER2, thus reducing the division of cancer cells.

The histological and ultrasound information along with clinical assessment are used to determine the stage of disease and used to guide patient management[36]. The TNM staging system of the American Joint Committee on Cancer uses both clinical and pathological information to suggest the prognosis of patients with cancer[37]. Patients are classified into different groups depending on the number and location of primary tumors (T), involvement of axillary lymph nodes (N), and distant metastasis (M) combine to make up stages 0 to IV **(Table 1)**[36,38]. In addition to determining stage, some pathological report also include clinical (c) and pathological (p) grading to indicate that a patient has locally recurring disease or systemic neoadjuvant or adjuvant therapy (y).

Primary Tumor (T)	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ, intraductal carcinoma, lobular carcinoma in situ
T1	Tumor ≤ 2 cm in greatest dimension
T1a	Tumor > 0.1 cm but ≤ 0.5 cm in greatest dimension
T1b	Tumor > 0.5 cm but ≤ 1cm in greatest dimension
T1c	Tumor > 1 cm but ≤ 2 cm in greatest dimension
T2	Tumor > 2 cm but ≤ 5 cm in greatest dimension
T3	Tumor 5 cm in greatest dimension
T4	Tumor of any size with direct extension to chest wall or skin only
T4a	Extension to chest wall
T4b	Edema or ulceration of the skin of the breast or satellite skin nodules confined to the same breast
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma
Regional Lymph Nodes (N)	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastases in one to three axillary lymph nodes and/or internal mammary nodes with metastases detected by SLN biopsy
N1a	Metastasis in one to three axillary lymph nodes, at least one metastasis greater than 2.0 nun
N1b	Metastasis in internal mammary nodes detected by SLN biopsy
N1c	Metastasis in one to three axillary lymph nodes and in internal mammary lymph nodes detected by SIN biopsy
N2	Metastasis in four to nine axillary lymph nodes or clinically detected, internal mammary lymph nodes in the absence of axillary lymph node metastases
N3	Metastases in 10 or more axillary lymph nodes or infraclavicular lymph nodes
Distant Metastasis (M)	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis, including metastasis to ipsilateral supraclavicular lymph nodes

Table 1: The American Joint Committee on Cancer TNM Staging System of Breast Cancer. Sentinel Lymph Node (SLN)

DCIS is primarily managed surgically either with breast conservation lumpectomy with or without radiation or total mastectomy. LCIS is treated with surgical excision to negative margins and adjuvant hormonal therapy such as tamoxifen, raloxifene, or aromatase inhibitors to reduce risk of tumor recurrence or progression of disease to invasive carcinoma. Early breast cancer contained within the breast and free of spread to the axillary lymph nodes is curable while metastatic disease is not. Stage 1 and II disease are considered early disease and managed with a combination of neoadjuvant chemotherapy, breast conservation surgery or mastectomy, and adjuvant radiation therapy and hormonal systemic therapy in a subset of hormone or epithelial growth factor positive breast cancer. Stage III disease also known as locally advanced disease is treated more aggressively with neoadjuvant systemic chemotherapy, total mastectomy and lymph node removal followed by radiation and hormone therapy. Given that stage IV disease is incurable, symptom management in attempt to prolong survival and improve quality of life with systemic chemotherapy, hormonal therapy, and radiation therapy is the main goal of the management[36].

Neoadjuvant Chemotherapy

Neoadjuvant chemotherapy (NACT) is used to describe chemotherapy given before surgery to reduce tumor size and increase the rate of breast conserving therapy. Historically, NACT has been used to treat early stage and locally advanced cancer, including inoperable and inflammatory breast cancers[39]. There are several advantages to use of NACT in locally advanced breast cancer treatment including reduction of tumor progression prior to surgery, decreased rate of cross-resistance to other chemotherapies,

and decrease in operation-related morbidity. NACT is associated with higher disease-free state and overall survival. Previous studies have shown that there is a direct correlation between an increased number of axillary lymph node metastases and worse overall prognosis without NACT when patients with similar nodal statuses were compare[40]. Additionally, it has been demonstrated that NACT downstages stage II/IIIA breast cancer, allowing for a cosmetically acceptable surgical approach. The overall response rate in each of the seven trials assessed was approximately 60 – 90% rate[40]. Other studies have shown a pathological complete response rate between 12 – 50% depending on characteristics of the tumor[41]. The efficacy of NACT is based on the type of regimen used. The individual regimen is determined based on molecular subtype, degree of tumor burden and risk of cancer recurrence. Currently, standard chemotherapy regimens for HER2- breast cancer include an anthracycline and a taxane given in sequence to reduce toxicity. Other agents like cyclophosphamide, carboplatin, methotrexate and/or fluorouracil are sometimes included in the regimen.

Anthracyclines: Mechanism of Action and Toxicity

Anthracyclines is a class of drugs extracted from the Streptomyces bacterium. They remain one the most effective chemotherapeutic agents used to treat several cancers[42]. The most important anthracyclines include doxorubicin, daunorubicin, epirubicin, and idarubicin with doxorubicin being the most commonly used agent in modern area[42]. Anthracyclines induce cytotoxicity and tumor killing by intercalating between DNA base pairs and inhibiting the activity of DNA topoisomerase II[43]. As such, the drug inhibits DNA and RNA synthesis and blocks cell division especially in highly

replicating cells like breast cancer cells. The bulky 4-ring structure of anthracyclines - a tetracycline molecule with anthraquinone backbone connected by a glyosidic linkage makes the intercalation between DNA base pairs effective. It has also being suggested that the quinone moiety of anthracyclines generate reactive oxidative species that result in oxidative stress which damages DNA and triggers cell apoptosis[43].

Although the mechanism of action of anthracycline makes it a very effective cytotoxic agent, the lack of specificity limits its use in humans. The two major side effects, both of which are dose dependent, include myelosuppression and cardiotoxicity[44]. Since the myelosuppression is reversible and can be combatted with therapeutic cytokines, cardiotoxicity is the major limitation for use of large doses of anthracyclines especially doxorubicin. Anthracycline-induced cardiotoxicity is dose dependent and cumulative and can occur both acutely and chronically. Acute toxicity occurs during or immediately after drug administration and results in aberrant electrical activity of the heart, hypotension, and vasodilation[44,45]. Chronic toxicity manifests as dilated cardiomyopathy, left ventricular dysfunction and eventually congestive heart failure. The oxidative stress and lipid peroxidation induced by anthracyclines has been implicated as the cause of the cardiac injury[44]. Anthracycline have a high affinity for cardiolipin, a phospholipid present in the mitochondria. This interaction causes cell injury and apoptosis induced by lipid peroxidation and reactive oxidative stress[44]. Cardiac tissues due to the relative high number of mitochondria are particularly susceptible to this reaction. Furthermore, cardiac tissue have low levels of anti-oxidant enzymes like catalase and superoxide dismutase limiting their defense mechanism against reactive oxidative species[46]. Given that the chronic toxicity of anthracyclines limits the use of

anthracyclines, several strategies have been attempted to minimize cardiotoxicity. In practice, there is a set lifetime maximum dose of 400 – 450 mg/m^2 recommended to prevent development of heart failure[47]. Finally, dexrazoxane is an iron chelating agent used to increase protection of cardiac tissues.

Taxanes: Mechanism of Action and Toxicity

Taxanes come from the diterpenes class of plants. Taxanes induce cell killing by disrupting microtubule function that are essential to cell division. By binding to GDP-bound tubulin in the microtubule, taxanes prevent the depolymerization of the mitotic spindle thus inhibiting cell division, trapping the cell cycle in G2 or M phase which eventually induce cell necrosis[48]. Docetaxel and paclitaxel are the major chemotherapeutic taxane agents used in the treatment of breast cancer. Since axonal degeneration is essential to nerve function, dose dependent neurotoxicity is the main side effect of taxane use[48].

In the treatment of early-stage and locally advanced breast cancer, several studies have shown that anthracycline-and taxane-containing neoadjuvant chemotherapy improves the response rate of breast cancer cells when compared to anthracycline-free neoadjuvant chemotherapy[10,49-51]. In some patients, anthracycline specifically doxorubicin is given before taxane, most commonly docetaxel and in others docetaxel is given before anthracycline. In HER2+ patients, this regimen is often followed with Transtuzumab. The administration of taxane before anthracycline has been associated

with improved pathological complete response, from 15% to 20%, compared with anthracycline-first sequence[52].

Breast Cancer Surgery

Surgical removal of the primary tumor remains a primary component of breast cancer treatment. Traditionally, radical mastectomy with complete removal of the breast and axillary lymph nodes was the surgical goal, however, over the past decade, conservation of the breast while maximizing treatment is becoming the mainstay surgical approach. As discussed above, in early-stage or locally advanced breast cancer, surgery often follows NACT. The use of NACT to shrink the tumor size makes breast-conserving surgery possible. In a study by the National Surgical Adjuvant Breast and Bowel Project (NSABP) randomizing patients with stage I or II disease with tumors less that 4cm to lumpectomy vs. lumpectomy plus radiation vs. mastectomy, there was no difference in overall survival between the groups. However, the rate of recurrence was significantly higher in the lumpectomy group with 39.2% developing disease after 20 years[53]. Ultimately, for tumors less than 5 cm, the decision should be patient-centered.

Mastectomy is the complete removal of breast tissue. Indications include locally advanced disease greater than 5cm, stage II-IV disease involving axillary lymph nodes or chest wall, persistent positive margins after breast-conserving surgery or inflammatory disease. There are several types of mastectomy based on how the surgery is performed and how much tissue is removed. In skin-sparing mastectomy, the inframammary fold and as much native skin as possible is preserved, only the breast tissue, nipple and areola

are removed. This approach is used when immediate breast reconstruction is planned[36]. Nipple-sparing mastectomy is very similar to skin-sparing. In this case, the breast tissue is removed while the breast skin and nipple are preserved. Nipple-sparing mastectomy is also performed with plans for immediate breast reconstruction. In both skin-sparing and nipple-spearing mastectomy, the nipple-areolar complex is assessed intraoperatively to ensure that there is no tumor involvement. In contrast, a simple mastectomy involved the excision of all breast tissue including the pectoralis major fascia, nipple, areola and screen with or without removal of axillary lymph nodes. Modified radical mastectomy as the name suggests is the most aggressive surgical approach, it combines simple mastectomy with removal of axillary lymph node dissection. It is used to treat higher stage, more invasive and inflammatory breast cancer[54].

Breast Reconstruction: Implant and Autologous Reconstruction

Immediate breast reconstruction is offered routinely to patients undergoing nipple-sparing or skin-sparing mastectomy as part of the management of breast cancer. Immediate breast reconstruction is patient-centered as it significantly reduces the negative psychological impacts of the body alteration in women who undergo mastectomy[16]. Several studies have demonstrated the oncological safety of immediate breast reconstruction, they found no increase in local or distant recurrence or changes in disease-free or overall survival in breast cancer patients, even in those with advanced disease[55-58]. The goal of breast reconstruction is to create the most naturally-appearing breast. The two major reconstructive approaches are implant-based and autologous tissue transfer. Implant-based techniques use prosthesis like silicone or saline while autologous

tissue technique uses the patient's tissues. Long-term satisfaction is higher with autologous reconstruction however, implants offer patients a safe, and less invasive option.

The use of implants for breast augmentation dates back to 1963 when silicone implants was first developed by Cronin[59]. The first attempt to use implant after mastectomy was in 1971. It usually follows a two-step approach. In the first step, tissue expanders are placed and gradually expanded until a desired size is reached. The second stage of the surgery replaces the tissue expander with a breast implant. Nipple reconstruction and/or mastectomy site revisions can be done at this time or, more commonly, at another revisionary surgery. Tissue expanders are either smooth versus textured. Smooth implants are mobile within the implant pocket while textured implants are immobile and adheres to the pocket[60]. Implants however, have been associated with higher surgical site infection rate, unnatural texture, pain associated with elevation of the underlying serratus muscle/fascia, capsular contracture, and risk of implant rupture. The complication rates are even higher in patients who undergo subsequent adjuvant radiation, with re-operation rates as high as 30%[61].

Autologous reconstruction uses tissue flaps from different areas of the body. Tissue flaps can be grouped into pedicle flaps and free flaps. Pedicle flaps are flaps that can be moved to the breast or chest wall while still being attached to its original blood supply, meanwhile, free flaps require anastomosis to the blood vessels in the chest wall for the flap to survive. Compared to implant reconstruction, autologous breast reconstruction is completely natural and gives a more natural feel. The absence of foreign

body also reduces the risk for infection and creates more long-lasting results. Finally, revision of skin defect is easier with autologous reconstruction compared to tissue expanders. The disadvantages of autologous reconstruction include a more technical and long-lasting surgery and increased donor site morbidity.

Tissue flaps can be obtained from several sites around the body; however, the most common reconstruction options are abdomen-based, latissimus dorsi, gluteal and thigh-based flaps. There are several options for abdomen-based flaps using muscles from the abdomen. The transverse rectus abdominis myocutaneous (TRAM) flap offers various advantages. It provides enough soft-tissue bulk for reconstruction of the breast mound. The TRAM flap can either be pedicled or free. The pedicled TRAM flap receives its blood supply from the superior epigastric artery tunneled through vessels within the rectus abdominis muscle. The main complications with this flap are anterior wall hernia and partial necrosis of the flap[60]. Free TRAM flaps receive blood supply from the inferior epigastric vascular pedicle; this allows more freedom for manipulating the tissue for better reconstructive outcome and minimal risk of necrosis. The deep inferior epigastric perforator (DIEP) flap is similar to the muscle-sparing free TRAM flap. However, the transmuscular perforators from the deep inferior epigastric artery perfuse the overlying skin which minimizes necrosis. Since no muscle is used, donor-site morbidity is also minimized[61]. Although DIEP flaps are most commonly used, it has been associated with higher risk of venous insufficiency, partial flap loss, and fat necrosis[62]. The latissimus dorsi flap is another reliable and commonly used flap. It gets robust blood supply from the thoracodorsal vessels making it advantageous in women at increased risk of wound complications following implant or TRAM flap reconstruction.

The potential impact that NACT have on breast reconstruction has been explored[15,16,18-20]. The study with the largest sample size by Mehrara et al. demonstrated that NACT is a predictor of minor post-operative complications particularly increased infection at the donor site[15]. Another study by Deutsch et al. revealed a 45% complication rate in patients who underwent immediate breast reconstruction after receiving NACT[19]. In contrast, a study by Abt et al. concluded that immediate reconstruction after NACT did not result in greater post-operative complication rate but actually reduced the rate of systemic morbidity following surgery[14]. Azzawi et al. in a retrospective analysis also had similar conclusion, they reported that although there was a higher incidence of minor surgical complications in patients who received NACT, these findings were not statistically significant[16].

STATEMENT OF PURPOSE

This thesis provides a summary of current breast cancer treatment modalities including neoadjuvant chemotherapy and mastectomy, and breast reconstruction techniques. It also examines in details the effects of specific NACT regimen on microvascular breast reconstruction complication outcomes. Lastly, it details our investigation on the potential effects of type of neoadjuvant chemotherapy agents, schedule, number of doses and time between last chemotherapy dose and surgery.

Specific Aims

1. To determine the effect of different anthracycline-taxane containing neoadjuvant chemotherapy regimens on post-complication outcomes in autologous breast reconstruction.
2. To provide objective data that informs patient counseling on neoadjuvant chemotherapy regimen.

METHODS

Student Contributions

Project question, literature search, chart review, data collection, statistical analysis, and thesis write-up were completed by the author of this thesis, Olamide Olawoyin, with contributions from other students including Sumarth Mehta (SM) and Fouad Chouairi (FC). SM contributed to chart review and data collection. FC provided advice on statistical analysis and contributed to the patient data base during the early stages of this project. Dr. Kyle Gabrick wrote the IRB approval and maintained the database. Dr. Lajos Pusztai provided expert guidance for the medical oncology component of this project. Dr. Michael Alperovich approved, supervised, and reviewed all data and thesis write-up.

Ethical Statement

This research was conducted according to the Yale University and Yale New-Haven Hospital ethical guidelines. Institutional Review Board approval was obtained prior to commencement of the project. Informed consent was obtained from patient at time of surgery as part of data collection for a larger data base. All patient data were deidentified and recorded in a confidential manner. Only patient data necessary for this project were recorded. Given the nature of the study, only women were included in the study. Minorities were included. There was no direct human subject involvement in this project. Animal subjects were not utilized in any part of this project.

Patient Population

We conducted an electronic chart review to identify patients who received neoadjuvant chemotherapy and immediate autologous breast reconstruction between 2013 and 2018 at the Yale New Haven Hospital. Data collected from the patients' chart included age, body mass index, race, stage of breast cancer, prior history of radiation therapy and abdominal surgery, smoking status, medical comorbidities including hypertension, diabetes, psychiatric history, American Society of Anesthesiologists score, and occurrence of minor and/or post-operative complications. The most common complications recorded were infections, abdominal or other donor-site dehiscence or necrosis, donor-site infections, partial or complete flap necrosis, abdominal bulge, operative hernia repair, arterial or venous flap insufficiency, breast hematoma, breast seroma, fat necrosis, and return to the operating room within 30 days. The incidence of fat necrosis was confirmed by ultrasound imaging. There were no cases of deep venous thrombosis / pulmonary embolism, nipple-areola complex necrosis, abdominal hematoma, or seroma in the cohort.

We also obtained neoadjuvant chemotherapy specific variables such as regimen, dosage, total length of treatment, and days between completion of chemotherapy and mastectomy/immediate autologous breast reconstruction. Finally, we collected data for complete blood count i.e. white blood cell count, absolute white blood cell count, absolute neutrophil count, absolute leukocyte count, and absolute neutrophil count/absolute leukocyte count ratio that were recorded at the time of last chemotherapy dose.

Details of Neoadjuvant Chemotherapy

We used the National Comprehensive Cancer Network Clinical Practice Guidelines (Version 4.2018) for Breast Cancer to classify neoadjuvant chemotherapy protocols. The types of chemotherapy regimen identified from the data set is detailed in the table below **(Table 2)**.

Regimen	Chemotherapy Content of Regimen (Dosage)
AC + wT	Adriamycin/Cyclophosphamide (every 3 weeks)
CMF	Cyclophosphamide, Methotrexate, Fluorouracil (every 3 weeks)
ddAC	Adriamycin/Cyclophosphamide (every 2 weeks)
ddAC + ddT	Adriamycin/Cyclophosphamide (every 2 weeks) + Paclitaxel (every 2 weeks)
ddAC + wT	Adriamycin/Cyclophosphamide (every 2 weeks) + Paclitaxel (every week)
ddTC	Docetaxel + Cyclophosphamide (every 2 weeks)
EC + wT	Epirubicin/Cyclophosphamide (every 3 weeks) + Paclitaxel (every week)
P	Carboplatin (every 3 weeks)
PwT + ddAC	Carboplatin (every 3 weeks) with Paclitaxel weekly + Adriamycin/Cyclophosphamide (every week)
wT	Paclitaxel (every week)
TC (HP)	Docetaxel/Cyclophosphamide (every 3 weeks), always with Transtuzumab (H) and Pertuzumab (P)
TC + A	Paclitaxel/Cyclophosphamide (every 3 weeks) + Adriamycin (every 2 weeks)
TP	Docetaxel/Carboplatin (every 3 weeks)
wT + CEF	Paclitaxel (every week) + Cyclophosphamide/Epirubicin/Fluorouracil (every 3 weeks)
wT + ddAC	Paclitaxel (every week) + Adriamycin/Cyclophosphamide (every 2 weeks)
wTwP + ddAC	Paclitaxel/Carboplatin (every week) + Adriamycin/Cyclophosphamide (every 2 weeks)

Table 2: Name of Neoadjuvant Chemotherapy Regimen and Content identified from Patients' Medical Electronic Records.

Generally, we grouped chemotherapy regimen based on the inclusion or exclusion of anthracycline. Grouped as first generation are anthracyline-containing regimens. Second generation regimen (T-AC) are defined as taxane-containing where taxane is administered before other agents such as cyclophosphamide and/or anthracycline. Finally, in third generation regimens (AC-T), administration of anthracycline is followed by taxane agent.

Statistical Analysis

We excluded patients with incomplete chemotherapy or reconstruction data from the analysis. Variable were stratified as described above and all statistical analyses were performed using IBM SPSS Version 25 (IBM Corp., Armonk, N.Y.). To analyze categorical variables, we performed a univariate analysis with chi-square test. P values for variables $n < 5$ are determined by Fisher's exact test, while Pearson's chi-square is used for variables $n > 5$. Continuous variables were analyzed using a univariate analysis with two-tailed t tests. The Shapiro-Wilk test was performed to test whether continuous variables followed a normal distribution. For continuous variables where a normal distribution could not be assumed, the Mann-Whitney U test was used to define the p-value, and the median was evaluated. Descriptive statistics for these variables are reported as mean ± SD, median, and interquartile range.

We controlled for demographics and comorbidities using a multivariate logistic regression analysis. Results were reported as frequency or mean ± SD for complication incidence. Univariate and multivariate p values were included as appropriate.

Multivariate odds ratios and 95% confidence intervals were reported for all regression models. We also calculated the areas under the receiver operative curve (ROC) to investigate the fitting behavior of the logistic regression models used. Statistical significance was set at $p < 0.05$ **(*p < 0.05, **p < 0.01)** for all analyses and areas under the curve (AUC) greater than 0.6 suggested a high performing logistic regression model.

RESULTS

Patient Demographics

In our review, a total of 467 patients underwent autologous breast reconstruction between 2013 and 2018 at the Yale New Haven Hospital. Of those, 100 patients who received neoadjuvant chemotherapy and met the inclusion criteria were included in further analyses. The mean age of those patients was 46.5 ± 9.6 years and mean body mass index of 30.6 ± 6.2 kg/m^2. 64% of patients were non-smokers, 30% were former smokers, and 6% were current smokers. Complete patient demographics and comorbidities are presented in **Table 3.**

Variables	Total
Age, y (mean ± SD)	46.5±9.6
BMI (mean ± SD)	30.6±6.2
Race n (%)	
Caucasian n (%)	71 (71%)
Hispanic n (%)	9 (9%)
African American n (%)	14 (14%)
Asian n (%)	3 (3%)
Other n (%)	3 (3%)
ASA n (%)	
II	46 (46.5%)
III	53 (53.5%)
Smoking Status n (%)	
Current Smoker	6 (6%)
Former Smoker	30 (30%)

Nonsmoker	64 (64%)
Comorbidities n (%)	
Hypertension	20 (20%)
Diabetes	7 (7%)
Prior Abdominal Surgery	51 (51%)
Breast Cancer Stage By Patient n (%)	
1	11 (12%)
2	25 (27.2%)
3	17 (18.5%)
3+	17 (18.5%)
Prophylactic and Stage 0 Contralateral breast	39 (42.4%)
History of chest irradiation	4 (4.0%)
Mastectomy Type Per Breast n (%)	
Modified Radical	18 (18.8%)
Simple/Skin Sparing	70 (72.9%)
Nipple Sparing (Prophylaxis for Contralateral breast)	8 (8.3%)

Table 3: Demographics, Comorbidities, and Operative Details for Autologous Breast Reconstruction Cohort.
Body mass index (BMI); American Society of Anesthesiologists (ASA).

Incidence of Any Complication

The incidence of minor or major complications was 48 out of 100 patients who met the inclusion criteria. There was no statistical difference in the mean age of patients with complications vs. those without complications (47.4±10.1 vs. 45.7±9.1, p-value = 0.384). However, the mean BMI of patients with complications was significantly higher than those without (32.0±7.1 vs. 29.3±5.0 (p-value = 0.029). There was no difference in the complication rates between former smokers and current smokers (p-value = 0.118). The presence of comorbidities such as hypertension, diabetes, and psychiatric comorbidities did not differ significantly between complication rates **(Table 4)**. Complication rates were higher for patients with high levels of comorbidities, such as ASA III (70.2% vs. 38.5%; p-value = 0.002). These patients also had a higher rate of 30 day return to the OR (p= 0.019) and higher incidence of complete mastectomy flap necrosis (p-value = 0.038). We did not observe a significant difference in complication rate among breast cancer stages or type of mastectomy.

Demographics by patient	**Presence of Complications (n=48)**	**Absence of Complications (n=52)**	**p-Value**
Age, y (mean ± SD)	47.4±10.1	45.7 ± 9.1	0.384
BMI (mean ± SD)	32.0±7.1	29.3±5.0	**0.029***
Race n (%)			
White n (%)	33 (68.8%)	38 (73.1%)	0.637
Hispanic n (%)	4 (8.3%)	5 (9.6%)	1.000
African American n (%)	8 (16.7%)	6 (11.5%)	0.456

Asian n (%)	1 (2.1%)	2 (3.8%)	1.000
Other n (%)	2 (4.2%)	1 (1.9%)	0.606
ASA n (%)			
II	14 (29.8%)	32 (61.5%)	**0.002****
III	33 (70.2%)	20 (38.5%)	**0.002****
Smoking Status n (%)			
Current Smoker	4 (8.3%)	2 (3.8%)	0.423
Former Smoker	18 (37.5%)	12 (23.1%)	0.118
Nonsmoker	26 (54.2%)	38 (73.1%)	**0.049***
Comorbidities n (%)			
Hypertension	12 (25%)	8 (15.4%)	0.230
Diabetes	4 (8.3%)	3 (5.8%)	0.707
Psychiatric Diagnosis	8 (16.7%)	6 (11.5%)	0.460
Prior Abdominal Surgery	24 (50%)	27 (51.9%)	0.848
Preop Radiation	2 (4.2%)	2 (3.8%)	1.000

Table 4: Univariate Analysis of Demographics and Comorbidities on Complication Rate
*p < 0.05, **p < 0.01. Significant values are denoted in bold.
Body Mass Index (BMI); American Society of Anesthesiologist (ASA) score.

Oncology and Chemotherapy Regimen

The average time between completion of chemotherapy and surgery was 35.1 ± 12.4 days and there was no time difference between patients with complications and those without. The average dose of chemotherapy agent was 12.9 ± 4.6 doses. In the group with complications, there were an average of 13.6±4.5 doses administered while 12.3±4.6 doses were administered in the group without complications (p-value = 0.179). We did not observe any statistically significant difference between patients' white blood cell (WBC), absolute neutrophil count (ANC), absolute lymphocyte count (ALC), or ratio

of ANC/ALC. In patients with complication, the WBC average was 6.6±2.4 and ANC/ALC ratio was 3.4±2.0 which was similar to 6.4±2.6 and 5.6±15.9 respectively in patients without complications **(Table 5)**.

	Total (n=100) Mean(1000/mm^3) ± SD	**Presence of Complications (n =48)**			**Absence of Complications (n=52)**			**P-Value**
		Mean ± SD	**Median**	**Q1 – Q3**	**Mean ± SD**	**Median**	**Q1 – Q3**	
Days between last chemo and surgery	73.8 ± 143.5	78.0 ± 151.8	31.0	27.0 – 42.5	70.0 ± 136.8	35.0	29.0 – 46.2	0.119
Total Dose	12.9 ± 4.6	13.6 ± 4.5	16.0	15.0 – 16.0	12.3 ± 4.6	16.0	8.0 – 16.0	0.179
CBC								
WBC	6.5 ± 2.5	6.6 ± 2.4	6.1	4.8 – 7.9	6.4 ± 2.6	5.8	4.6 – 7.2	0.453
ANC	4.4 ± 2.3	4.3 ± 2.1	3.7	3.0 – 4.8	4.2 ± 2.4	3.3	3.0 – 4.9	0.468
ALC	1.4 ± 0.6	1.4 ± 0.5	1.2	1.0 – 1.7	1.4 ± 0.6	1.4	1.0 – 1.6	0.789
ANC/ALC	4.6 ± 11.7	3.4 ± 2.0	2.8	1.9 – 4.4	5.6 ± 15.9	2.5	1.7 – 3.7	0.362

Table 5: Univariate Analysis of Chemotherapy Outcomes on Complication Rate *p < 0.05, **p < 0.01. Significant values are denoted in bold.
Complete Blood Count (CBC); White Blood Cell (WBC); Absolute Neutrophil Count (ANC); Absolute Lymphocyte Count (ALC).

Order of Anthracycline and Taxane Administration in Regimen

Of the 100 patients who met inclusion criteria for our study, eighty patients received NACT with an anthracyline-containing regimen. Seventy-nine of those patients received two or more agents containing both taxane and anthracycline i.e. 2nd or 3rd generation regimen. Thirty-four patients were administered taxane first (2nd generation regimen) and forty-five patients received taxane second in the sequence (3rd generation).

One patient received only anthracyline and cyclophosphamide and the remaining twenty patients were administered a single agent of anthracycline only (1st generation).

In general, among the patients who received anthracyline-containing regimen, forty-one had post-operative complications and thirty-nine had none. In the group of patients with one or more complications, 43.8% received a taxane-first regimen compared to 39.6% when taxane was administered second. In contrast, in patients with no complications, 25% received taxane first while 50% received taxane second in the sequence. Patients who received taxane first had a statistically significant higher rate of complication(s) (p-value = 0.048). Hormone therapy did not have any significant impact on post-operative complications **(Table 6).**

Variables	Total (n=100)	Presence of Complications (n=48)	Absence of Complications (n=52)	P-Value
Anthracycline n (%)	80 (80%)	41 (85.4%)	39 (75%)	0.193
Order of Chemotherapy				
Single agent Chemotherapy	21 (21%)	8 (16.7%)	13 (25.0%)	0.307
Combination – Taxane second	45 (45%)	19 (39.6%)	26 (50.0%)	0.296
Taxane first - combination	34 (34%)	21 (43.8%)	13 (25.0%)	**0.048***
Hormone Therapy n (%)				
Transtuzumab	40 (40%)	18 (37.5%)	22 (42.3%)	0.624
Pertuzumab	29 (29%)	12 (25%)	17 (32.7%)	0.397

Table 6: Univariate Analysis of Chemotherapy Regimen on Complication Rates *p < 0.05, **p < 0.01. Significant values are denoted in bold.

Stratification of Incidence of One of More Complications

All the patient in our cohort underwent either DIEP or muscle-sparing TRAM autologous breast reconstruction. Since some patients underwent prophylactic contralateral breast mastectomy and reconstruction, there was a total of 163 breasts among the 100 patients in our cohort. We did not observe a statistically significant difference in incidence of complications between breasts who underwent DIEP flap reconstruction (n = 137) or muscle-sparing TRAM flaps (n = 26). Post-operative complication rate also did not differ with number of perforators among breasts. Due to unrecorded data in some of the patients, we report flap weight for thirty-nine of the 163 breasts. In these breasts, we did not observe a statistically significant difference between the mean flap weight of breasts with complication (810.3gm ± 456.6gm) compared to breasts without complications (618.5gm ± 195.1gm), p-value = = 0.183. Furthermore, post-operative radiation to the flap did not seem to impact complication rate (p-value = 0.309). Fifty-eight breasts underwent post-operative radiation to the flap. We performed univariate analysis for all covariates described above and detailed in **Table 7.**

	Total	Presence of Complications			Absence of Complications			P-Value
		Mean ± SD (n)	Median	Q1 – Q3	Mean ± SD (n)	Median	Q1 – Q3	
# of Perforators per flap	[a]n = 139	2.2 ± 0.97 (43)	2.0	2.0 – 3.0	2.1 ± 0.8 (96)	2.0	2.0 – 3.0	0.913
Weight of flap (g)	[a]n = 39	810 ± 456 (17)	727	440 - 956	6 18 ± 195 (22)	678	435 - 808	0.183

	Total (n = 163)	Presence of Complications (n=56)	Absence of Complications (n=107)	P-Value
Type of Flap				0.899
DIEP	137 (84%)	47 (83.9%)	90 (84%)	
MS-TRAM	26 (16%)	9 (16%)	17 (15.9%)	
Axillary dissection	56 (34.3%)	19 (33.9%)	37 (34.6%)	0.838
Post-op radiation	58 (35.6%)	23 (39.3%)	35 (32.7%)	0.309
Bilateral mastectomy	132 (80.9%)	44 (78.6%)	88 (82.2%)	0.768

Table 7: Univariate Analysis of Flap Covariates on Complication Rate $^{*}p < 0.05$, $^{**}p < 0.01$ (by breast) [a] Total number ( ) differ due to missing data. Significant values are denoted in bold.
Deep Inferior Epigastric Perforator (DIEP); Muscle-Sparing Transverse Rectus Abdominis Myocutaneous (MS-TRAM).

We conducted univariate analysis of axillary lymph node dissection in order to control for any potential effects of invasive or metastatic disease **(Table 7).** Fifty-six patients underwent axillary lymph node dissection and there was no significant impact on rate of post-operative complications among these patients (p-value = 0.838).

We performed a multivariate binary logistic regression analysis to control for confounding variables such as BMI, ASA, diabetes, post-operative radiation, axillary lymph node dissection, unilateral vs. bilateral flap reconstruction, DIEP vs. muscle-sparing TRAM flap, and number of perforators per flap. We observed that higher BMI was associated with higher rate of complications (p-value = 0.020; odd ratio = 1.185)

(Table 8). More importantly, we observed a positive statistically significant association with administration of taxane first and rate of post-operative complications. Patients who received taxane first had higher rates of complications even after controlling for the aforementioned potential confounding variables (p-value = 0.012, OR 3.521) **(Table 8)**.

Adverse Effects	**OR (95% CI)**	**p-Value**
T – AC	3.521 (1.317 – 9.416)	**0.012***
BMI	1.185 (1.019 – 1.245)	**0.020***
ASA	1.762 (1.061 – 6.499)	**0.037***
Diabetes	1.409 (0.115– 11.650)	0.901
Post – op Radiation	2.667 (0.980 – 7.052)	0.055
Axillary LN Dissection	0.946 (0.331 – 2.472)	0.844
Unilateral vs Bilateral Mastectomy	1.471 (0.384– 4.263)	0.688
DIEP vs MS-TRAM	0.334 (0.086 – 1.307)	0.115
Number of Perforators	1.278 (0.854 – 2.525)	0.165

Table 8: Multivariate Binary Logistic Analysis of Chemotherapy Regimen on Complication Rates Controlling for BMI, ASA, Post-op Radiation, Axillary LN Dissection, Microvascular type, Perfusion and number of Perforator. *p < 0.05, **p < 0.01 (by breast). Significant values are denoted in bold.
Taxane – Anthracycline/Cyclophosphamide (T - AC)

Using a logistic regression model to determine the effect of taxane-first therapy on complication rates, AUC was calculated as 0.760; 95% CI 0.666 - 0.853, p * 0.0001 (Figure 1), which indicates that the model performed well overall **(Figure 1)**. This suggests that the taxane-first regimen is associated with complications in 76% of cases.

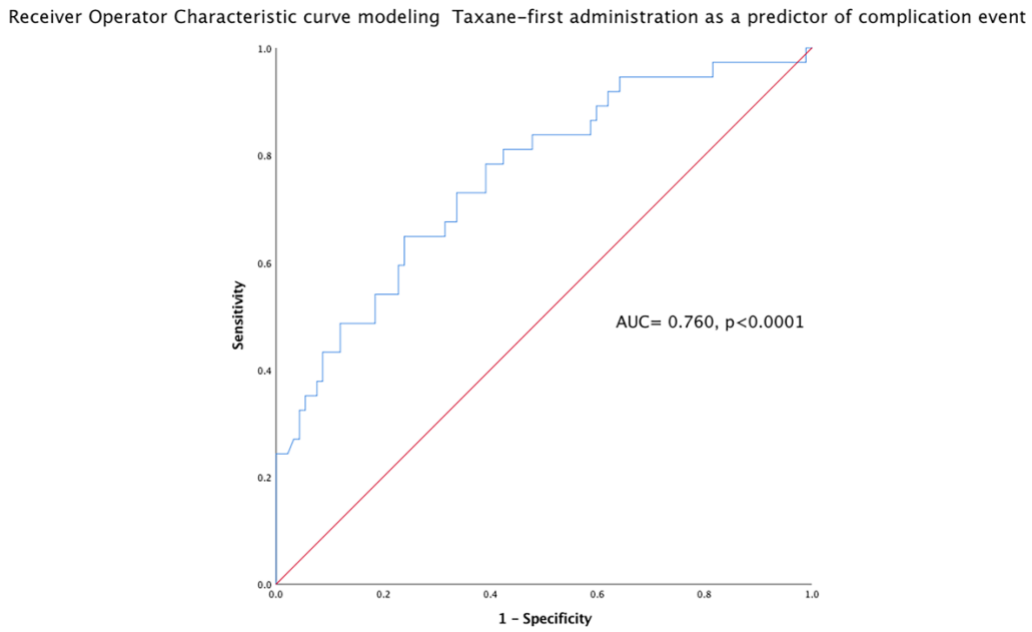

Figure 1: Receiver operator characteristic curve modeling taxane-first administration as a predictor of complication event.

Fat Necrosis

Upon further stratification of type of complications, we observed that the incidence of fat necrosis differed significantly based on sequence of taxane administration. Univariate analysis showed that 28.3% of flaps in patients administered taxane first had fat necrosis post-operatively, but only 14.6% when taxane was administered second in the sequence (p=0.035) **(Table 9)**.

Variables	Taxane first (n=34)	Remaining Cohort (n = 66)	p-value
Any complication n (%)	21 (61.8%)	27 (40.9%)	**0.048***
Microvascular Complications n(%)			
Abdominal Hematoma	0	0	
Abdominal Seroma	0	0	
Insufficiency			
Arterial	1 (3.0%)	1 (1.5%)	1.000
Venous	1 (3.0%)	1 (1.5%)	1.000
Donor Site Infection	0	0	
Abdominal Dehiscence			0.356
Dressing Changes	3 (8.8%)	2 (3%)	
OR Repair	0 (0%)	1 (1.5%)	
Abdominal Site Necrosis			0.770
Dressing Changes	0 (0%)	1 (1.5%)	
OR Repair	1 (2.9%)	2 (3%)	
Bulge	3 (8.8%)	1 (1.5%)	0.113
Operative Hernia Repair	3 (8.8%)	1 (1.5%)	0.113
Flap complications n(%)	**Taxane First (n=60)**	**Remaining Cohort (n = 103)**	**p-value**
DVT/PE n (%)	0	0	
Mastectomy Flap Necrosis n (%)			0.676
Partial	2 (3.3%)	5 (4.9%)	
Complete	2 (3.3%)	6 (5.8%)	
NAC Necrosis	0	0	
Fat Necrosis	17 (28.3%)	15 (14.6%)	**0.035***
Infection	3 (3.3%)	2 (1.9%)	0.360
Breast Hematoma	1 (1.6%)	2(1.9%)	1.000
Breast Seroma	1 (1.6%)	3 (2.9%)	1.000
Return to OR in 30days	7 (11.6%)	10 (9.7%)	0.740

Table 9: Univariate Analysis of Order of Taxane Administration on Complication Rates. *p < 0.05, **p < 0.01. Significant values are denoted in bold.

On multivariate binary logistic regression controlling for potential confounders, administration of taxane first was associated with a 2.5-fold increase in the risk of fat necrosis (p-value = 0.040; OR = 2.481) **(Table 10)**.

Adverse Effects	**OR (95% CI)**	**p-Value**
T – AC	2.481 (1.041 – 5.914)	**0.040***
BMI	1.019 (0.958 – 1.084)	0.552
Diabetes	0.714 (0.073– 7.036)	0.773
Post – op Radiation	2.457 (0.951 – 6.350)	0.063
Axillary LN Dissection	0.890 (0.343 – 2.307)	0.811
Unilateral vs Bilateral Mastectomy	1.407 (0.458– 4.317)	0.551
DIEP vs TRAM	0.767 (0.236 –2.499)	0.660

Table 10: Multivariate Binary Logistic Analysis of Chemotherapy Regimen on Fat Necrosis Controlling for BMI, Diabetes, Post-op Radiation, Axillary LN Dissection, Microvascular type. $^{*}p < 0.05$, $^{**}p < 0.01$. Significant values are denoted in bold.

In the logistic regression model evaluating the effect of taxane-first regimen specifically on fat necrosis, the AUC was estimated to be 0.635; 95% CI 0.514 – 0.756, $p < 0.05$ **(Figure 2)**, indicating an overall satisfactory model performance**.** Overall, the AUC estimates that taxane-first regimen is associated with fat necrosis in 64% of cases.

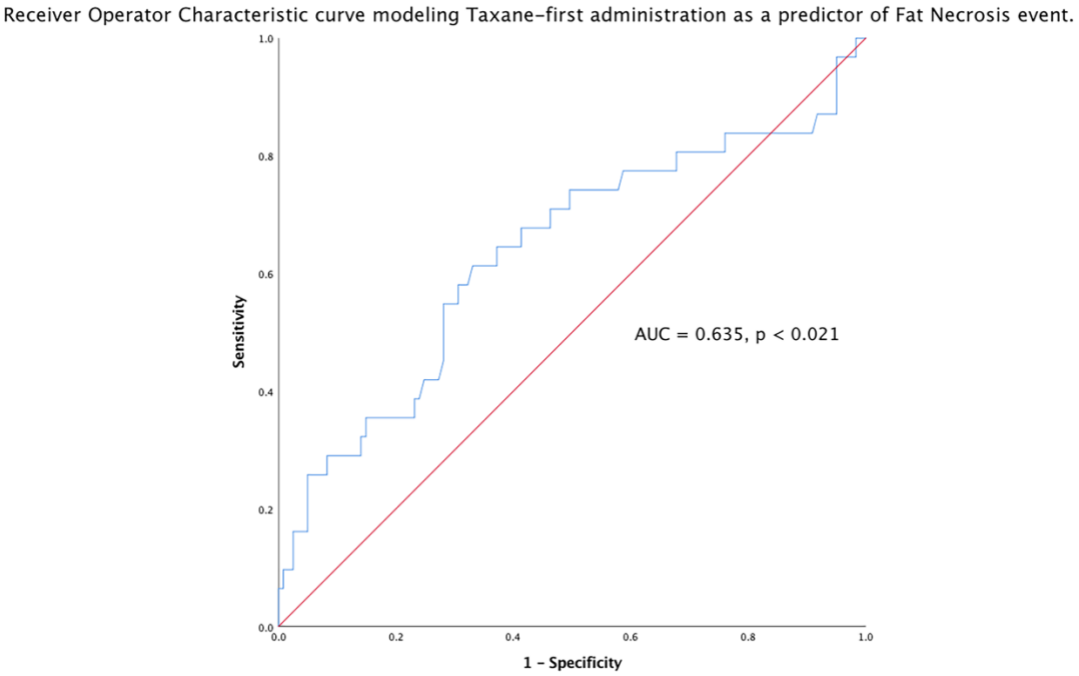

Figure 2: Receiver operator characteristic curve modeling taxane-first administration as a predictor of fat necrosis event.

Furthermore, the diagnosis of fat necrosis was made between 9 and 895 days after surgery and the median number of days was 129.50 days **(Table 11)**. Patients who received taxane first and those who did not have statistically significant differences in the time between surgery and fat necrosis diagnosis (p-value = 0.895). Finally, a total of five patients with fat necrosis were operated on after confirming the diagnosis on ultrasound, two of whom had received taxane first and three who had not. The size of the necrotic fat tissue did not differ between patients who received taxane first (2.54cm ±1.54 cm) when compared to the patients who did not (2.39cm ± 0.98cm), p-value = 0.813 **(Table 11)**.

	T – AC			Remaining Cohort			P-Value
	Mean ± SD (n)	**Median**	**Q1 – Q3**	**Mean ± SD (n)**	**Median**	**Q1 – Q3**	
# of perforators	2.4 ± 1.1 (14)	2.00	1.8 – 3.0	2.0 ± 0.9 (11)	2.0	1.0 – 2.0	0.429
Weight of flap (g)	679 ± 536 (6)	440	405 - 909	833 ± 221 (6)	879	656 - 1020	0.077
# of days between surgery and fat necrosis	277 ± 197 (17)	209	147 - 386	361 ± 454 (15)	245	69 - 378	0.895
Size of fat necrosis by ultrasound (cm)	2.5 ± 1.5 (9)	2.0	1.4 – 4.3	2.4 ± 1.0 (6)	2.2	1.6 – 3.3	0.813
Return to OR for Fat Necrosis Removal	2 (5)	-	-	3 (5)	-	-	0.522

Table 11: Univariate Analysis of Fat Necrosis by Order of Taxane. $^{*}p < 0.05$, $^{**}p < 0.01$. Significant values are denoted in bold.

Intraoperative Details

In order to evaluate for adequate perfusion in the flap, indocyanine green imaging (ICG) was used prior to insetting of the flap by the reconstructive surgery. ICG is a water soluble cyanine dye that binds to blood proteins such as albumin and lipoprotein. The spectral properties of ICG in the near infrared range makes it a suitable surrogate marker for angiography and evaluation of tissue perfusion[63,64]. It has a peak absorption in serum between 790 and 805 mm and peak emission at 835 mm allowing for penetration in blood and improved visualization during surgery[63]. We recorded ICG for 127 of the 163 flaps in our study. In addition to using ICG to assess for adequate perfusion, the reconstructive surgeon also clinically evaluated rate of bleeding during de-epithelization. Poorly perfusing flaps were not used. Any watershed areas with questionable perfusion were

debrided on the abdomen before the flap was transferred to the chest. ICG was not used to assess flap perfusion after microsurgical anastomosis.

DISCUSSION

Neoadjuvant chemotherapy (NACT) reduces the tumor size and the risk of axillary metastases. Several controlled trials have shown that NACT have similar recurrence rates, disease-free, and overall survival rates as adjuvant chemotherapy[7,8]. Since NACT shrinks tumor size, the administration offers additional benefit for treatment of early-stage and locally advanced breast cancer[12]. By shrinking the tumor size, NACT makes breast conserving therapy in treatment of advanced breast cancer possible, allowing for immediate autologous breast reconstruction[8-10].

Patients with HER2 negative disease often receive anthracycline-based regimens i.e. doxorubicin (Adriamycin) or epirubicin and cyclophosphamide, preceded or followed by a taxane (docetaxel or paclitaxel)[65] with Carboplatin also sometimes used. Patients with contraindications, particularly those with cardiac disease are often offered an anthracycline-free alternative, which includes taxane and cyclophosphamide or carboplatin[66]. A few patients receive cyclophosphamide, methotrexate and fluorouracil-based regimen[67]. Anthracycline free regimen are termed 1st generation, anthracycline and taxane containing regimen are 2nd generation and anthracycline, taxane, and hormone therapies (Transtuzumab and or Pertuzumab) are 3rd generation[68]. Several studies have shown the benefit of anthracycline-containing (2nd or 3rd generation) over anthracycline-free (1st generation) regimen in improving tumor response rate[10,49-51]. A study demonstrates that the administration of taxane before anthracycline was associated with a significant improvement in tumor response[69]. The administration of taxane before anthracycline was also associated with significant improvement in tumor response[69].

In this study, we took a detailed approach to analyze the effect of neoadjuvant chemotherapy regimen on post-operative microvascular breast reconstruction outcomes. For the first time in literature, we report a significant association between administration of taxane before anthracycline and higher incidence of minor post-operative complication, specifically fat necrosis both on univariate and multivariate analysis. On further analysis of types of post-operative complications, we confirm previous findings that have demonstrated that major complications are infrequent.

Advances made in the surgical techniques of autologous (free flap) breast reconstruction over the years has significantly reduced donor site morbidities[70]. However, across all options for free flap (Transverse Rectus Abdominis Muscle, Deep Inferior Epigastric Perforator flap, Superficial Inferior Epigastric Artery flap, Superior Gluteal Artery Perforator, and Profunda Artery Perforator flap), fat necrosis persist as a minor complication[70]. Our finding confirms previous findings that have demonstrated an association between fat necrosis and neoadjuvant chemotherapy[15,20,21]. Similar to these studies, we observed that comorbidities such as affect post-operative outcomes.

Although ASA is not the main focus of this study, this study, like others[71-73], demonstrate that higher ASA score of III correlates with higher incidence of perioperative complications. ASA score, based on five classes (I to V) is an assessment of patient's overall preoperative health. ASA I indicates a normal healthy patient, II indicates a patient with mild systemic disease and III indicates a patient with severe systemic disease[74]. None of the patients in our study had ASA IV or V which denote a patient with life-threatening disease[74]. Administering taxane first remained significantly

associated with higher complication rate after controlling for ASA as a confounding variable.

Exploring the mechanism of action of taxane offers an insight into why it may affect fat necrosis when administered first. Taxanes stabilize the mitotic spindle, inhibit cell division, and induce necrosis by disrupting the disassembly of microtubules[75]. Taxanes are potentially more potent due to the effect on the tumor microenvironment, reduced clearance, and their pharmacokinetic interaction with anthracylines[76]. Few studies have explored the effects of the sequence of taxane administration on post-operative outcomes. Earl et al. in their exploration of the role of adding gemcitabine to paclitaxel and epirubicin, an anthracyline and cyclophosphamide, they demonstrate that although this NACT combo did not improve the pathological complete response, sequencing NACT administration so that taxane is received before anthracyclines improve the pathological response rate defined as absence of invasive cancer in the breast cancer and axillary lymph nodes[69]. Shi et al. in their investigation of the effect of chemotherapy on expression of heat shock protein 27, a protein whose overexpression leads to anthracyline resistant, report that paclitaxel (a taxane) when given before doxorubicin (an anthracycline) is more effective at clearing breast cancer cells with high expression of high shock protein compare with doxorubicin-paclitaxel sequence[77]. Furthermore, Taghian et al. report that paclitaxel could enhance anthracycline's cytotoxic impact on tumor cells by increasing interstitial fluid pressure and oxygenation within the tumor microenvironment[78]. These investigations demonstrate that taxane-first NACT regimen may potentiate tumor killing. Possibly, the higher potency of taxane-first

administration increase cytotoxicity to the extent that it ultimately induces necrosis in normal fatty breast tissue.

Post-operative fat necrosis has also been associated with radiation to the flap in some studies. However, other studies similar to ours do not find this association. Mirzabeigi et al. in their investigation of patients undergoing immediate autologous breast reconstruction and adjuvant radiation found a higher incidence of fat necrosis in irradiated patients[79]. Garvey et al. also reports similar findings, concluding that radiation after DIEP and MS-TRAM based breast reconstructions had higher rates of flap necrosis[80]. In contrast, Clarke-Pearson et al. reported no clinically significant fat necrosis observed in either irradiated or nonirradiated DIEP flaps undergone during the immediate DIEP reconstruction between 2009 and 2011[81]. An additional retrospective study of 183 patients undergoing immediate flap reconstructions by Crisera et al. found that postoperative radiation therapy did not predict fat necrosis[82]. According to Taghizadeh et al. and Kronowitz, this discrepancy may be due to the timing of breast reconstruction[83]. The relatively small size of our study may have also contributed to this discrepancy.

Furthermore, we show that dosage, the time interval between completion of NACT and surgery, and complete blood count do not affect post-operative outcomes. This was unexpected since taxane and anthracycline have been shown to affect the immunological health of the patient. In fact, one of the most devastating side effects of anthracyclines is myelosuppression and neutropenia due to cytotoxicity to the bone marrow[44]. The effect of NACT on blood cell count was important to explore because NACT has been shown to delay wound healing especially when administered two weeks

prior to surgery[19]. Absolute neutrophil count (ANC) less 500 cells/mm^3 have been shown clinically to lead to worse wound healing outcomes[84]. The institutional protocol at Yale-New Haven Hospital for this cohort has been to delay surgery until ANC recovers and is above 1.0 cells x 1000/mm^3. The higher ANC of the patients in our study before surgery possibly explains why we observed no significant post-operative wound healing complications.

The limitations of this study include a small sample size. We were underpowered to detect differences in complications based on each NACT regimen. Missing data in the electronic medical records of some patients in our database led to a small sample size. This also prevented us from doing more detailed analysis of the effect of factors like number of perforators used and flap perfusion prior to inset. In addition, as our study is a retrospective study, a prospective randomized case-controlled study needs to be done to confirm our claim. Despite these limitations, we were able to reveal that the sequence of anthracycline-containing NACT regimen plays a role, albeit, minor in the post-operative complication rate of microvascular breast reconstruction.

CONCLUSION

The administration of taxane first in an anthracycline-containing NACT regimen contributes to minor post-operative autologous breast reconstruction complications. Specifically, taxane first administration correlated with more incidence of fat necrosis. However, the dosage of chemotherapy, number of days between NACT completion and surgery, and number of circulating immune cells had no effect on post-operative outcomes. Our report has the potential to inform the sequence of NACT administration, thus reducing post- operative complication outcomes. This report also provides objective data that can help counsel patients and delineate expectations for their immediate autologous breast reconstruction. The benefit of taxane-first in improving tumor outcome should be taken into consideration when making clinical decisions as this may outweigh the risks of post-operative outcomes.

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