Pathological Complete Response in early-stage HER-2 positive breast cancer patients, receiving neoadjuvant chemotherapy/trastuzumab, in a single breast unit in Johannesburg

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Dedication:

I would like to dedicate this paper to Nicola, Tonia, Stefanie, Vincenzo, Alessia, Prabashni, Simona and Nicola Jr, who have been the greatest reason for my successes. They continue to be by my side on this journey, understanding and supporting the sacrifices that have to be made in pursuit of my drive and ambition towards surgical excellence. With their constant love and support there are no limits to what I can achieve.

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1. Abstract

Background

HER-2 positive breast cancers receiving neoadjuvant therapy containing Trastuzumab show higher rates of pathological complete response, correlating to better clinical outcomes. Trastuzumab is not available in the neoadjuvant setting in the public healthcare system in South Africa.

Methods

This study evaluated factors affecting pCR in early-stage HER-2 positive breast cancers. We retrospectively analysed data of 102 patients with early-stage HER-2 positive breast cancer who completed neoadjuvant trastuzumab/chemotherapy. The cohort was analysed for total pCR and looked at factors that may affect pCR (tumor size, menopausal status, hormone receptor status, Ki-67 levels, and nodal status).

Results

The pCR rate for the entire cohort was 58.82%. Factors associated with a higher pCR were ER receptor status (ER negative= 82.05%, ER positive 44.4%, P<0.00018), PR receptor status (PR negative=75.41% vs PR positive 34.15%, P< 0.00003). The Ki67 <14% was 46.15%, Ki67 14-30% was 51.11% and Ki67> 30% was 70.45% (P<0.037). Univariate analysis showed a significant difference in pCR relating to ER/PR/HER-2 grouping (ER/PR positive= 63.64%, Triple positive= 34.15%, Enriched= 82.05%; P<0.00007).

Conclusions:

This data highlights the importance of characterising the different subtypes of HER-2 positive early breast cancers and the association with pCR, in keeping with the international guidelines. Higher pCR rates were attained in HER-2 enriched subtypes. Currently, in South Africa, trastuzumab is not readily available in the public healthcare sector. This study emphasizes the need for trastuzumab to be made available to all patients with HER-2 positive breast cancers in the neoadjuvant setting in South Africa.

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4. List of Abbreviations

EML: Essential medications list

ER: Oestrogen receptor

ESMO: European society for medical oncology

HER-2: Human epidermal growth factor 2

HR: Hormone receptor

NAC: Neoadjuvant chemotherapy

NCCN: National comprehensive cancer network

pCR: pathological complete response

PR: Progesterone receptor

5. Introduction

According to the South African National Cancer Registry (2014)[1] breast cancer is the most common newly diagnosed cancer in women in South Africa, making up 21.78% of new cancer diagnoses across all race groups, with an incidence of 33.35 per 100 000. In 2020, breast cancer was ranked as the 5th leading cause of cancer-related death in women worldwide, with GLOBOCAN estimates of 684 996 female deaths relating to the disease.[2]

Up to two-thirds of breast cancer-related deaths occur in low- and middle-income countries due to late presentation and inadequate access to treatment.[3] By 2030 there is predicted to be a shift of the global burden of the disease towards the low- and middle-income countries.[4] In sub-Saharan Africa, breast cancer is the second leading cause of cancer-related death in women [5], highlighting the need for better treatment for breast cancer to improve survival.

Pathological complete response (pCR) is defined as the absence of invasive cancer, excluding in-situ cancer, in breast tissue and axillary nodes (ypT0 ypN0) on resection specimens following neoadjuvant chemotherapy/trastuzumab.[6] A study by Li *et al*. in 2018 found an overall pCR rate of 33% in patients with HER-2 positive disease, receiving NAC (neoadjuvant chemotherapy) compared to 6% pCR rate in patients with Luminal B cancers. The same study further compared the efficacy of different chemotherapeutic regimens, finding that patients receiving docetaxel, carboplatin, and trastuzumab (TCH) had the best pCR rate when compared to other NAC regimens.[7] Human epidermal growth factor receptor-2 (HER-2) is overexpressed in 15-30% of primary breast cancers. [8] HER-2 positive breast cancers have a more rapid and aggressive course, with an increased chance of presentation as locally advanced and/or metastatic disease. [9] Dong and colleagues (2018)[9] found that patients with hormone receptor (HR) -negative, HER-2 positive disease were more likely to attain pCR than patients with HR-positive, HER-2 positive cancers, following neoadjuvant chemotherapy/trastuzumab. This highlights the need for effective treatment of early breast cancers identified as HER-2 positive.

Treatment with HER-2 targeted monoclonal antibodies has been shown to be beneficial in attaining pCR in HER-2 positive breast cancers.[8] A pooled analysis by Cortazar *et al*. (2014) showed that T1 and T2 breast cancers had 18.3% and 19.9% pCR rates when receiving neoadjuvant chemotherapy. Furthermore, when comparing the HR status and HER-2 status, the following findings were reported (Table 1).

Table 1: Pathological complete response by tumor subtype[6]

Tumour Subtype	pCR
HER-2 negative, HR-positive	7.5%
HER-2 positive, HR-positive (receiving trastuzumab)	30.9%
HER-2 positive, HR-positive (not receiving trastuzumab)	18.3%
HER-2 positive, HR-negative (receiving trastuzumab)	50.3%
HER-2 positive, HR-negative (not receiving trastuzumab)	30.2%

After statistical analysis it was concluded that pCR was positively associated with a better long-term prognosis.6 HER-2 positive, HR-negative disease treated with neoadjuvant therapy containing trastuzumab showed the highest rates of pCR. In contrast, HER-2 positive, HR-positive disease treated with neoadjuvant therapy containing trastuzumab had considerably

higher rates of pCR than those treated with regimens not containing trastuzumab.[6] This highlights the importance of determining rates of pCR in patients with HER-2 positive breast cancer receiving NAC, as trastuzumab/NAC increases the rate of pCR in these cancers and improves long-term prognosis for this subgroup of patients.

This study investigated overall pCR and factors affecting pCR in patients with early-stage HER-2 positive breast cancers receiving neoadjuvant chemotherapy/trastuzumab.

6. Materials and Methods

This retrospective study included 102 early-stage HER-2 positive breast cancer patients treated with neoadjuvant therapy at the Milpark Hospital Breast Unit. Neoadjuvant therapy included anthracycline and/or taxane based chemotherapy/trastuzumab.

Pathological complete response (pCR) is defined in this study as the absence of invasive cancer, excluding in-situ cancer, in breast tissue and axillary nodes (ypT0ypN0) on resection specimens following neoadjuvant chemotherapy/trastuzumab.[6]

The study population consisted of T1/T2 tumors, clinically node-negative (patients with positive sentinel lymph node biopsies were included), and HER-2 positive cancers (ICH/SISH). Excluded from the study were patients with locally advanced and metastatic disease, clinically node-positive disease and those with HER-2 negative disease.

Data was collected from existing databases at Milpark Hospital Breast Unit and Medical Oncology Centre of Rosebank.

Statistical methods:

Categorical variables were described using frequency, percentages and charts. Continuous variables were described using mean +/-standard deviation or median and interquartile range (if not normally distributed). The rate (and 95% confidence interval) of pCR was calculated by dividing all the histological samples with pCR by the total study sample.

The rate of pCR was compared among menopausal status, primary tumor size, ER receptor status, PR receptor status, nodal status, Ki-67, and chemotherapeutic regimen. The Pearson's Chi square test (Chi^2) was used to compare different categorical variables. Multivariable logistic regression using ER, PR, and HER-2 status as independent variables, with pCR as the dependent variable was done. ROC curve method was used to set cutpoints to analyse the low, intermediate, and high Ki-67 subgroups pertaining to pCR. A two-tailed test of the hypothesis was assumed and the test of significance was set at a p-value <0.05. All data analysis was performed using NCSS 11 Statistical Software (2016, NCSS, LLC, Kaysville, Utah, USA, ncss.com/software/ncss).

7. Results

The pCR rate for the entire cohort was 58.82% (59/102). On univariate analysis: Primary tumor size found pCR present in 68.75% (22/32) patients with T1 tumors and 54.3% (38/70) patients with T2 tumors which was not statistically significant (Chi2= 1.8969 p= 0.168427) (Figure 1a). Premenopausal patients showed a 50% (18/36) pCR rate, while in postmenopausal patients, 63.64% (42/66) had pCR, which was also not statistically significant (Chi2 1.2030, P<0.272) (Figure 1b). ER receptor status showed a pCR rate of 82.05% (32/39) in ER-negative patients and 44.4% (28/63) in ER-positive patients, which was statistically significant (Chi2 14.0650, P<0.00018) (Figure 1c). PR receptor status showed a pCR rate of 75.41% (46/61) in PR-negative patients and 34.15% (14/41) in PR-positive patients, which was statistically significant (Chi2 17.2364, P< 0.00003)(Figure 1d). N0 patients had a pCR rate of 65.08%

(41/63) vs. 48.72% (19/39) in N1 patients, which was not statistically significant (Chi20,0308, P< 0.860)(Figure 1e).

Figure 1a. Tumor Size.

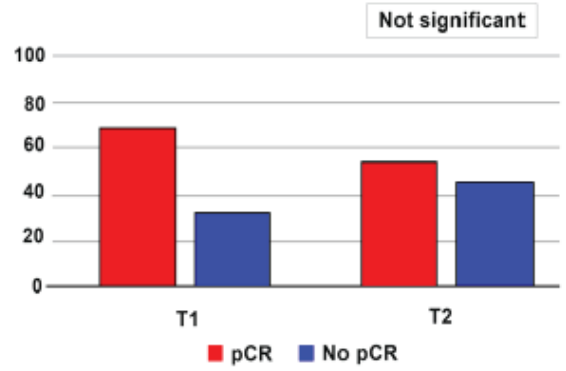


Figure 1b. Menopausal Status.

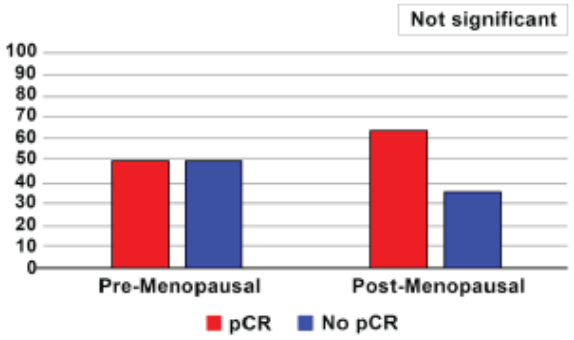


Figure 1c. ER Status.

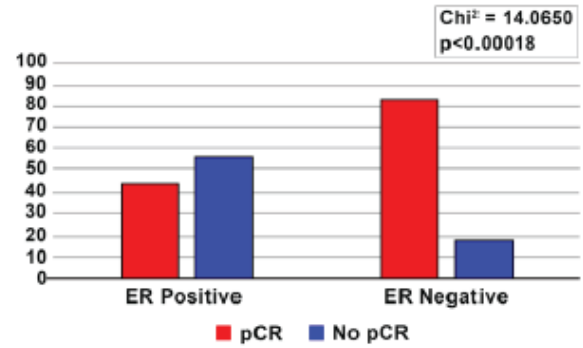


Figure 1d. PR Status.

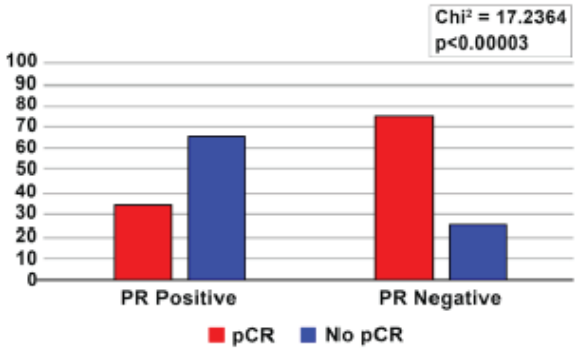


Figure 1e. Nodal Status.

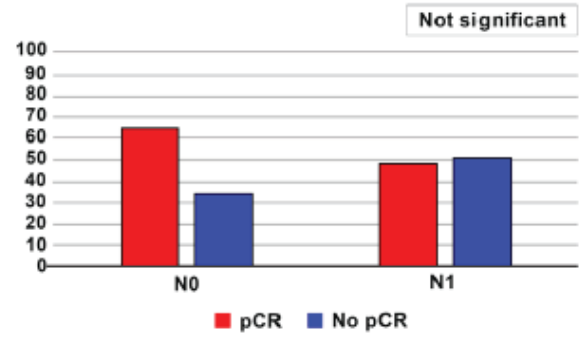
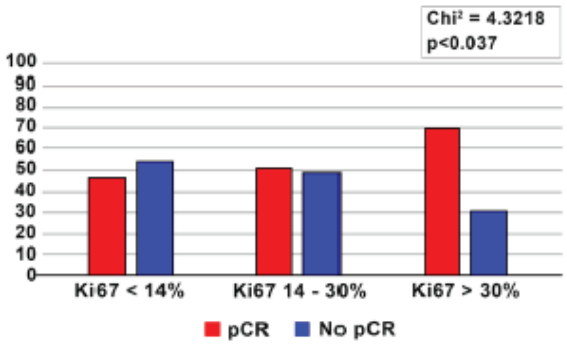


Figure 2. Ki67.



Results of the Ki-67 analysis showed that pCR for Ki-67 <14% was 46.15% (6/13), Ki-67 14-30% was 51.11% (23/45) and Ki-67> 30% was 70.45% (31/44) which proved to be statistically significant (Chi2 4.3218 P<0.037) (Figure 2). There was a significant difference in pCR rate in relation to the ER/PR/HER-2 grouping. The HER-2 enriched subgroup had pCR rate of 82.05%, ER/PR-positive 63.64% and triple positive 34.15%, (Chi2 19.2056, P<0.00007).

Pathological complete response was attained in 54.55% (18/33) of doxorubicin based chemotherapeutic regimens. Regimens not containing doxorubicin had a pCR rate of 60.87% (42/69); this difference was not statistically significant (Chi2 0,3686, P< 0,54377). A logistic regression model was developed including the following variables: ER positive, PR-positive, Ki-67<30, HER-2 enriched, and triple positive. Low Ki-67 and triple positive phenotype were

significant with P-values of 0.04608 and 0.00023 respectively. The other variables lost significance in the logistic regression model.

8. Discussion

This study was undertaken to evaluate the pCR rate of the chemotherapy/trastuzumab based neoadjuvant treatment in the South African insured population. South Africa has a 2-tiered health care system, including the state healthcare sector (82.8%) and private healthcare sector for the insured population (17.2%) as per the 2019 General Household Survey conducted by the South African department of statistics.[10] The majority of the health care insurance plans have cover for trastuzumab based neoadjuvant therapy, while the state healthcare system covers trastuzumab only in the adjuvant setting. The state healthcare sector budget is budget R248 billion. 11 The annual average spend is R4480 per person in the state healthcare sector vs R17225 per person in private healthcare sector highlighting healthcare disparities in South Africa.[11] Neoadjuvant chemotherapy is used to attain pCR in early-stage HER-2 positive breast cancers.[9] Traditionally, endpoints of treatment in this setting include event-free survival (EFS), disease-free survival (DFS), or overall survival (OS).[7] Multiple randomised control trials have proven the relationship between pCR and improved overall survival.[12] The use of pCR as a surrogate endpoint of treatment offers short term benefits in that it allows for the opportunity to monitor the disease progression in a much more acute fashion, providing insight into the responsiveness of the cancer to systemic therapy, as well as being associated with better overall prognosis.[13] The total pCR rate in our study was 58%. This compares well with other studies involving NAC of HER-2 positive disease.[6,9,14,16] (Table 2) HER-2 positive/HR-negative and HER-2 positive/HR-positive breast cancers appear to be biologically distinct entities in terms of their disease profile.[7] Dong and colleagues in 2018[9] found that women with HER-2 positive/HR-negative breast cancers were more likely to attain pCR than those with HER-2 positive/HR-positive cancers. Our data support these findings. We found an 82.05% rate of pCR in HER-2 positive ER negative cancers, and a rate of 75.41% in HER-2 positive PR negative cancers, which were found to be statistically significant. This compares favourably to the rates reported in the NeoSphere trial who reported an overall pCR rate of 63.2% for HR negative disease vs. 26% for HR-positive disease.[15] Our multivariate analysis further demonstrated this point in showing that the HER-2 enriched subtype of cancers obtained statistically significantly higher rates of pCR than the ER/PR positive or triple positive subgroups.

Table 2 pCR rate: Overall vs HER-2 Enriched vs ER/PR positive vs Triple positive

pCR (%)	Overall	HER-2 enriched	ER/PR Positive	Triple positive
Bellomo et al., 2022	58.82%	82.05%	63.64%	34.15%
Buzdar et al., 2005[14]	65.2%	70%	61.5%	Not reported
Gianni et al., 2012[15]	29%	63.2%	26%	Not reported
Cortazar et al.,2014[6]	46.4%	50.3%	30.9%	Not reported
Alba et al., 2016[16]	26%	49%	24.2%	Not reported
Dong et al., 2018[9]	45%	56.75%	14.29%	Not reported
Esteva et al., 2019[19]	47%*	Not reported	Not reported	Not reported
WSG-ADAPT Trial[20,21]	Not reported	36.6% ** 90.5% ***	30.8%	Not reported

*pCR rate for Stage I/IIA disease in this study
** Trastuzumab + pertuzumab
***Trastuzumab + pertuzumab + paclitaxel

Results based on ROC curves analysis showed Ki-67 cutpoints with the highest sensitivity and specificity were 14, 30, and >30. Based on these cutpoints (low <14; intermediate 14-30; high >30), pCR was observed in 15% of cancers with low Ki-67, 51.11% in cancers with intermediate Ki-67 and 70.45% in cancers with high Ki-67 values, which was found to be statistically significant. A study by Alba and colleagues in 2016 used a ROC curve analysis to separate their study population into high (>50) and low (<50) Ki-67 values. Pathological complete response was present in 45% (Ki- 67<50) and 63% (Ki-67>50) of HER-2 positive/ER-negative cancers, 23.5 % (Ki-67<50) and 27.3% (Ki-67 >50) in HER-2 positive/HR-positive cancers.16 The total obtained pCR for HER-2 positive cancers in the study were 32.96% (Ki-67 <50) and 45.45% (Ki-67 >50).[15] The study concluded that a Ki-67 index >50 could be considered an independent predictor of pCR after neoadjuvant therapy[16], a finding that is in keeping with our data (Table 3).

Table 3: Ki-67

	Bellomo *et al*.	Alba *et al.*
Ki-67 <15, pCR	15%	
Ki-67 15-30, pCR	51.11%	
Ki-67 >30, pCR	70.45%	
Ki-67 <50, pCR		32.96%
Ki-67 >50, pCR		45.5%

Factors found not to show statistically significant differences to pCR in our study were: initial tumor size, menopausal status, nodal status, and use of doxorubicin vs no doxorubicin in the neoadjuvant chemotherapy regimens. Addition of pertuzumab in neoadjuvant setting for HER-2 positive breast cancers is associated with increased rates of pCR.[15] Future directions in this field include evaluation of dual therapy with pertuzumab, trastuzumab and chemotherapy in this patient population. This analysis should include enriched, ER/PR-positive, triple positive as well as Ki-67 subgroups. Incorporation of gene-expression profiling in this patient population might give additional insights into the subgroup of patients that are likely to have improved outcomes with this treatment.

9. Conclusions

In conclusion this data confirms the importance of characterising early-stage HER-2 positive breast cancers as a potential means of predicting tumor response to neoadjuvant therapy and predicting pCR. The current standard of care in HER-2 positive breast cancer is neoadjuvant treatment with chemotherapy and herceptin as per the 2021 NCCN Breast Cancer Guideline, as well as the 2020 ESMO Early breast cancer guideline.[17,18]. Our data confirms the importance of this standard of care as HER-2 enriched subtypes attained a higher pCR rate than the other subtypes, similar to other published studies in this field (table 2). Currently, in South Africa, trastuzumab is not available on the essential medicine list (EML) for use in the neoadjuvant setting in the state health care sector, highlighting the healthcare disparities in this system.

10. Limitations

Limitations of this study include that it was a single centre retrospective cohort of patients in the private healthcare sector, and that pCR rates were not compared to the cohort of patients

in the public healthcare sector who do not receive neoadjuvant trastuzumab. Further research comparing these two groups of patients is needed in order to motivate for trastuzumab to be to be made available to all patients with early-stage HER-2 positive disease in the neoadjuvant setting in South Africa.

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12. Appendix

Approved Protocol

Pathological Complete Response in early-stage HER-2 positive breast cancer patients, receiving neoadjuvant chemotherapy/trastuzumab, in a single breast unit in Johannesburg

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1. LIST OF ABBREVIATIONS

NAC: Neoadjuvant Chemotherapy

FISH: Fluorescence in-situ hybridization

SISH: Silver-enhanced in-situ hybridization

HR: Hormone receptor

pCR: Pathological complete response

HER-2: Human epidermal growth factor receptor-2

TCH: Docetaxel/Carboplatin/Trastuzumab

ACT: Doxorubicin/Cyclophosphamide/Paclitaxel

2. ABSTRACT

Breast cancer is one of the most common cancers in women throughout the world, with an incidence of 43/100 000. In sub-Saharan Africa, breast cancer is the second leading cause of cancer related death in women.

HER-2 positive breast cancers have a more rapid and aggressive course, with increased chance of presentation as locally advanced and/or metastatic disease. HER-2 positive breast cancers receiving neoadjuvant therapy containing Trastuzumab show higher rates of pCR, which correlate to better clinical outcomes.

Using the existing Milpark Breast Unit database, a retrospective cross-sectional analysis will be conducted to determine the prevalence of pathological complete response in patients with early-stage HER-2 positive breast cancer receiving neoadjuvant chemotherapy/Trastuzumab. Furthermore, we will analyse the prevalence of pCR with regards to tumor size, hormone receptor status and type of chemotherapeutic regimen.

3. INTRODUCTION

Breast cancer is one of the most common cancers in women throughout the world, with an incidence of 43/100 000 (Singh *et al*. 2016). In 2012, breast cancer was ranked as the 5th leading cause of cancer related death in women worldwide, with GLOBOCAN estimates of 522 000 or 13/100 000 female deaths relating to the disease (Ferlay *et al*. 2012). According to the South African National Cancer Registry (2014) breast cancer is the most common newly diagnosed cancer in women in South Africa, making up 21.78% of new cancer diagnoses across all race groups, with an incidence of 33.35 per 100 000.

Up to two thirds of breast cancer related deaths are estimated to occur in low- and middle-income countries due to late presentation and inadequate access to treatment (Saxena *et al* 2012). By 2030 there is predicted to be a shift of the global burden of the disease towards the low- and middle- income countries (Singh *et al*. 2016). In sub-Saharan Africa breast cancer is the second leading cause of cancer related death in women (Dickens *et al.* 2014), highlighting the need for improvements in breast cancer treatment in order to improve survival.

Clinically, breast cancer can be divided into three broad categories: early breast cancer, locally advanced breast cancer and metastatic breast cancer (Pathak *et al*. 2018). Breast cancer is a heterogeneous disease, with differentiation into the different subtypes an important tool in prognostication and treatment strategies (Dickens *et al*. 2016). Furthermore, breast cancer can be stratified according to molecular subtypes, listed below:

1. Luminal A are hormone receptor positive (ER and/or PR positive), HER-2 negative and with low Ki-67 levels. These tend to be low grade (Grade I/II), slow growing tumors with good prognoses.
2. Luminal B are hormone receptor positive (ER and/or PR positive), HER-2 negative/positive and with high levels of Ki-67. Tumours in this subgroup are higher

grade tumours (Grade II/III), and are often associated with BRCA-2 mutations. These tumours run a more aggressive course and are associated with worse clinical outcomes.

3. HER-2 enriched (non-luminal) are hormone receptor negative and HER-2 positive. This subgroup has a more rapid clinical progression, however often have good response to monoclonal antibody therapies specifically targeted at HER-2 protein, such as Herceptin (Trastuzumab), if treated early.
4. Normal-like or basal-like breast cancers are minimally differentiated, hormone receptor negative, HER-2 negative and with low levels of Ki-67. This subgroup is associated with lymphocyte infiltration and are a heterogenous subgroup of cancers.
5. Triple negative cancers are hormone receptor negative (ER and PR negative), HER-2 negative, and more commonly associated with BRCA1 gene mutations. These tumours are usually little differentiated, Grade II/III tumours with rapid clinical course, poor response to neoadjuvant chemotherapy and worse clinical outcomes.

   (Boisserie-Lacroix *et al*. 2014)

Pathological complete response (pCR) is defined as the absence of invasive cancer, excluding in-situ cancer, in breast tissue and axillary nodes (ypT0 ypN0) on resection specimens following neoadjuvant chemotherapy and Trastuzumab (Cortazar *et al*. 2014).

Neoadjuvant chemotherapy (NAC) was initially proposed by Frie and colleagues in the 1980s and since then much work has been done looking at the efficacy of different neoadjuvant regimens. A study by Li *et al* published in 2018 found that there was an overall pCR rate of 33% in patients with HER-2 positive breast cancer, receiving NAC as compared to 6% pCR rate in patients with Luminal B cancers. The same study further compared the efficacy of different chemotherapeutic regimens, finding that patients receiving docetaxel, carboplatin and Trastuzumab (TCH) had the best pCR rate when compared to other NAC regimens (Li *et al.* 2018).

Human epidermal growth factor receptor-2 (HER-2) is overexpressed in 15-30% of primary breast cancers (Awada *et al.*2016). Overexpression of the HER-2 gene is associated with a more rapid clinical course and poorer prognosis (Awada *et al.*2016). HER-2 status is determined by overexpression of the HER-2 protein on immunohistochemical staining (3+) or by fluorescence in-situ hybridization (FISH) on histological samples (Zheng *e al*. 2018). An alternative to FISH is silver-enhanced in-situ hybridization (SISH). A study by Papouchado and colleagues in 2010 showed that there was a 98.9% concordance between FISH and SISH, with the added advantages of slides being able to be interpreted by conventional light field microscopy, not requiring fluorescence microscopy.

HER-2 overexpression is associated with poor regulation of cellular proliferation, adhesion, migration and differentiation (Zheng *e al*. 2018). HER-2 positive breast cancers have a more rapid and aggressive course, with increased chance of presentation as locally advanced and/or metastatic disease, thus highlighting the need for effective treatment of early breast cancers identified as HER-2 positive (Dong *et al*. 2018). A study by Dong and colleagues (2018) found that patients with HR negative, HER-2 positive disease were more likely to attain pCR than patients with HR positive, HER-2 positive cancers, following neoadjuvant chemotherapy.

Treatment with HER-2 targeted monoclonal antibodies has shown much benefit in attaining pCR in HER-2 positive breast cancers (Awada *et al.*2016). A recent pooled analysis by Cortazar *et al.* (2014) showed that T1 and T2 breast cancers had 18.3% and 19.9% pCR rate when receiving neoadjuvant chemotherapy. Furthermore, when comparing the HR status and HER-2 status the following findings were reported:

Tumour Subtype	pCR
HER-2 negative, HR positive	7.5%
HER-2 positive, HR positive (receiving Trastuzumab)	30.9%
HER-2 positive, HR positive (not receiving Trastuzumab)	18.3%

HER-2 positive, HR negative (receiving Trastuzumab)	50.3%
HER-2 positive, HR negative (not receiving Trastuzumab)	30.2%

After statistical analysis it was concluded that pCR was positively associated with better long-term prognosis (Cortazar *et al.* 2014). HER-2 positive, HR negative disease treated with neoadjuvant therapy containing Trastuzumab showed the highest rates of pCR, while HER-2 positive, HR positive disease treated with neoadjuvant therapy containing Trastuzumab had considerably higher rates of pCR than those treated with regimens not containing Trastuzumab. This goes to highlight the importance of determining rates of pCR in patients with HER-2 positive breast cancer receiving neoadjuvant therapy, as addition of Trastuzumab significantly increases the rate of pCR in these cancers and improves long term prognosis for this subgroup of patients.

4. AIM

The aim of this study is to investigate the prevalence of pathological complete response in patients with early-stage HER-2 positive breast cancers, receiving neoadjuvant chemotherapy/Trastuzumab, from a single breast unit in Johannesburg.

4.1 STUDY OBJECTIVES

1. To determine the incidence of pathological complete response in early-stage HER-2 positive breast cancers receiving neoadjuvant chemotherapy and Trastuzumab.
2. To compare the incidence of pathological complete response in T1 and T2 tumors.
3. To compare the incidence of pathological complete response in hormone receptor positive, HER-2 positive and hormone receptor negative, HER-2 positive early-stage breast cancers.

4. To compare incidence of pathological complete response of the different chemotherapeutic regimens (TCH vs ACT).
5. To evaluate the factors affecting pathological complete response in early-stage HER-2 positive breast cancers receiving neoadjuvant chemotherapy/Trastuzumab.

5. METHODS

5.1 Study design

Retrospective cross-sectional study on patients from the Milpark Breast Unit Database

5.2 Site of study

Milpark Hospital, Breast Unit and The Medical Oncology Centre of Rosebank

5.3 Study population

Patients with histologically diagnosed breast cancer, confirmed to be HER-2 positive, seen by the breast unit at Milpark Hospital or seen at The Medical Oncology Centre of Rosebank and then referred to the breast unit at Milpark Hospital.

5.4 Sample Preparation

Histological examination of mastectomy specimens has been carried out by AmPath Laboratories (Milpark). The laboratory uses accepted international standards in preparation and evaluation of histological specimens. The specimen is received, measured and weighed. Ink margins are evaluated to establish tumor distance to margins. The nipple and areolar are sectioned and the breast divided into quadrants. Thereafter the entire breast is sectioned into 2 cm thick slices and examined. The specimen is then examined and all macroscopic abnormalities described in detail. The tumor is sectioned into at least 3 sections or sections of 1cm diameter, including the centre and periphery of the tumor and adjacent tissue which is then assessed microscopically. A report is then generated by the anatomical pathologists once the specimen has been assessed. (Fitzgibbons *et al*. 2000)

5.4 Sampling

Data will be used from the existing database at Milpark Hospital and The Medical Oncology Centre of Rosebank.

Time period of review will be determined in a retrospective fashion according to the number of cases required, starting with 2020, and working backwards until a statistically significant number of cases have been collected.

5.5 Sample size

Sample size was calculated using a likelihood ratio test for comparing two independent proportions from power and sample size, with a confidence interval of 0.05 and a power of 0.8 (STATA statistical package version 15)

The calculated sample size required to ensure statistical significance is 80 (P_1= 0.183, P_2= 0.503, assuming a 1:1 ratio). We will aim for a sample size of 95 patients to make up for patients that might not fit the final criteria for the study.

5.6 Inclusion criteria

- T1+T2 tumors
- Clinically N_0
- Radiological studies done
- Up front sentinel lymph node biopsy done
- HER-2 positive confirmed (IHC/SISH)
- Hormone receptor positive and negative

5.7 Exclusion criteria

- Locally advanced and metastatic disease
- Clinically node positive disease
- HER-2 negative disease

5.8 Measuring tool and data collection

Data collection Sheet (Appendix 1)

5.9 Sources of bias

Data collected from a single hospital site which may not accurately represent nationwide demographics.

6. DATA ANALYSIS

Categorical variables will be described using frequency, percentages and charts. Continuous variables will be described using mean +/-Standard deviation or median and interquartile range (if not normally distributed). The prevalence (and 95% confidence interval) of the pCR will be calculated by dividing all the histological samples with pCR by the total study sample. The prevalence of pCR will be compared among tumor size, hormone receptor status and chemotherapeutic regimen using the Pearson's chi square test. Univariable and multivariable logistic regression will be conducted with pCR status as the outcome. Two tailed test of the hypothesis will be assumed and the test of significance will be set at p-value <0.05

7. BENEFITS

This study will show the prevalence of pathological complete response in early-stage HER-2 positive breast cancers, in patients receiving neoadjuvant chemotherapy/Trastuzumab. Historically, HER-2 positive cancers have been more aggressive and with poorer outcomes. With the addition of Trastuzumab rates of pCR have increased in international studies. pCR has a direct correlation to long term patient outcome. This study will form the framework for future studies in which long term patient outcomes can be correlated with the rate of pCR.

8. ETHICS

Unconditional ethical approval granted

Protocol No M200121 MED 19-11-142

9. TIMELINE

	Jan-Feb 2019	March-April 2019	12/06/ 2019	07/11 2019	Aug 2020-Sept 2020	Sept-Oct 2020	Oct-Dec 2020
Literature review	**X**						
Preparing Protocol		**X**					
Protocol Assessment			**X**				
Ethics Application				**X**			
Data Collection					**X**		
Data Analysis						**X**	
Writing up paper							**X**

10. COST

No major expenses are expected

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APPENDIX 1

Data Collection Sheet

Pathological Complete Response in Early-stage HER-2 positive Breast Cancer patients, receiving neoadjuvant chemotherapy/Trastuzumab, in a single breast unit in Johannesburg

A. Bellomo MMED (Surg)

Data Collection sheet

Study Patient Number:

Patient Demographics:

Age (years)		
Race		
Menopausal status	Premenopausal	Postmenopausal

Date of Diagnosis:

Initial Cancer staging:

T	
N	
M	

Stage:

Core Biopsy Histology (Date/Histology number/Site):

Lymphovascular invasion: YES/ NO

FNA:

Initial Tumor Size:

Receptor status:

HER-2	IHC			SISH	
	1+	2+	3+	Negative	Positive
ER	Negative			Positive	
PR	Negative			Positive	
Ki-67 (%)					

Radiological investigations:

U\S Breast:

Mammogram:

MRI:

Sentinel Lymph Node Biopsy:

Chemotherapy Regimen:

Date of commencement of treatment:

Date of completion of treatment:

	Cycles		
	T	C	H
TCH			
	A	C	T
ACT			
Other			
Herceptin	9 weeks	1 year	

Date of Primary Surgery:

Type of Surgery Done: Lumpectomy/Mastectomy/Skin Sparing

Histology from Primary Surgery: (Ductal/Lobular)

Radiation: YES/NO

Endocrine Therapy: YES/NO

Type:

Date of commencement:

Date stopped:

pCR: Yes/No

Residual disease: Tumor/Lymph Node

Recurrence: YES/NO

Date of Recurrence:

Mortality: YES/NO

Cause and date of Death: Treatment Toxicity/Cancer related/Non-Cancer Related (DD/MM/YYYY)