The Effect of Metformin When Combined With Neoadjuvant Chemotherapy in Breast Cancer Patients

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The effect of metformin when combined with neoadjuvant chemotherapy in breast cancer patients

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Abstract

Background: Metformin has been used to treat type 2 Diabetes Mellitus since long time. It has two proposed anti-neoplastic mechanisms, direct (insulin-independent) and indirect (insulin-dependent) actions.

Purpose: To assess the effect of Metformin on pathological response when combined with neoadjuvant chemotherapy in breast cancer.

Material and Methods: A prospective study included stage II, III non-diabetic breast cancer patients who received neoadjuvant chemotherapy in our center during the period from May 2017 to March 2019. 59 patients met our inclusion criteria and completed the study, 27 patients received 850mg Metformin every 12 hrs with chemotherapy (group A), 32 patients received chemotherapy without Metformin (group B). Pathological response was assessed by Chevallier classification and residual cancer burden score (RCB).

Results: Both groups were well balanced regarding base line characteristics. The results of our study showed that the rate of pathological complete response (pCR) was 14.8% in group (A) vs. 6.3% in group (B) with a P-value of 0.39. RCB class 3 was 40.7% in group (A) Vs. 68.8% in group (B) which was statistically significant with a (P-value of 0.031). Patients with triple-positive histology who had RCB class 3 were only (14.3%) in group (A) versus (60%) in group B. Patients with body mass index (BMI) ≥25 who had RCB 3 were 40% and 66.7% in group (A) and (B) respectively.

Conclusion: Metformin may increase the pCR especially in patients with BMI≥25 and patients with triple-positive histology, a larger phase III study is needed to confirm this finding.

Keywords: Metformin, Breast cancer, Chemotherapy**,** Neoadjuvant treatment, pathological response.

Introduction

Breast cancer can be presented at an early stage or locally advanced breast cancer (LABC)[1]. The incidence of LABC is not common in rich and high income-countries. According to US National Cancer Database and European CONCORD study, LABC represents 9.6% and 4% of breast cancer patients, respectively. However, this percent jump to 60% in developing and low-income countries[2-5]. The prognosis of LABC is as in early breast cancer, it depends on clinical stage, tumor grade, and expression of ER, PR, and HER-2 receptors. But, the most important prognostic tool for long-term survival is the pathological complete response (pCR) after neoadjuvant chemotherapy (NACT). pCR is now accepted to be used in all cases where chemotherapy was indicated at diagnosis before surgery for the aim of improving DFS and OS[5]. Many systems were validated to assess the pCR. In general, the systems define two groups; a group with pathologic complete response (pCR) and a group with little or no response. The classes of partial response in different systems range from 1 to 4[6] In almost all systems, a pCR requires invasive carcinoma absence in the breast. Likewise, the presence of residual ductal carcinoma in situ (DCIS) is considered also pCR as it doesn't alter survival.[7] In systems that include lymph nodes, the nodes must also be negative for a pCR.[7]

Therefore, many trials were conducted to study the effect of adding different agents to neoadjuvant chemotherapy aiming for increasing the rates of pCR. One of the proposed agents that might have an effect on the pCR rate is Metformin.

Diabetes has been found to be a risk factor for breast cancer in some but not all studies.[8] A large meta-analysis that was done to demonstrate the association between breast cancer and diabetes showed that females with type 2 diabetes mellitus (T2DM) had a 23% higher risk of developing

breast cancer in comparison to non-diabetic females[9]. The common factor linking diabetes, obesity, and metabolic syndrome to cancer could be the insulin resistance and the resulting hyperinsulinemia associated with these conditions.[10-12]. Insulin and insulin-like growth factor (IGF) receptors form a complex network of cell surface receptors, and all function to mediate insulin and IGF responses.[13] Most cancer cells have insulin and IGF-1 receptors. The insulin receptors are able to stimulate cancer cell proliferation and metastasis besides its metabolic function[14].

Metformin is a biguanide derivative and has been used for nearly a century to treat T2DM[9, 15-17]. The proposed mechanisms of the anti-neoplastic effect of metformin involve both direct (insulin-independent) and indirect (insulin-dependent) actions of the drug as illustrated in" Fig (1) in the supplementary materials". First, the indirect effects of metformin are associated with reduced circulating insulin levels. [18]As insulin has mitogenic and anti-apoptotic effects, the reduction in its level may be important to the anticancer mechanisms of metformin, especially in cancers associated with obesity and hyperinsulinemia, such as those of the breast and colon.[18] Moreover, certain cancers, like breast cancer, express high levels of the insulin receptor (IR), and high insulin level is associated with breast cancer recurrence and death.[19-21]. Second, the direct effects of metformin include activation of AMP-activated protein kinase (AMPK) via phosphorylation on Thr172 by the tumor suppressor liver kinase B1 (LKB1) and a subsequent reduction in mammalian target of rapamycin (mTOR) signaling, protein synthesis and cell proliferation[18, 22]

Material and Methods

We did a prospective randomized study that included 118 patients who were eligible for neoadjuvant chemotherapy during the period from May 2017 to March 2019 as illustrated in fig

(1). The main inclusion criteria were pathologically proven stage II or III breast cancer females' ≥20 years, non-diabetic with adequate hematological, hepatic, and renal functions. Using Echocardiography, the ejection fraction had to be ≥50%. The main exclusion criteria were pregnancy, congestive heart failure including, severe hepatic or renal impairment, and patients with known hypersensitivity to metformin.

A written informed consent was taken from all patients after approval from the ethics committee in our University "The Ethics Committee of the Faculty of Medicine-Alexandria University is constituted and operates according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern EC operation. We used AJCC 8th edition to assess both clinical and pathological stages of the patients.

Treatment plan:

All patients received (1) AC regimen (4 cycles every 21 days): Adriamycin 60mg/m^2 IV push, and Cyclophosphamide 600 mg/m^2 IV infusion over 20-60 min, or (2) AC-T regimen same AC regimen followed by paclitaxel 80mg/m^2 every week for 12 weeks. Group (A) received a chemotherapy regimen either AC or AC-T as mentioned above with Metformin 850mg starting once daily for one week to adapt gastrointestinal symptoms then twice daily and Metformin was stopped at least 48 hours before anesthesia according to FAD guidelines to avoid the risk of lactic acidosis[23], Group (B) received chemotherapy only, the same regimens as group (A) but without Metformin.

Tolerability and side effects

We followed the tolerability and recorded the side effects of treatment protocol using Common Terminology Criteria for Adverse Events v3.0 (CTCAE)[24].

Clinical response

Clinical response was evaluated using **RECIST criteria.**[25] All patients underwent clinical examinations before each cycle with the exclusion of any patients who developed progressive diseases. All patients had surgery 3-6 weeks after completing chemotherapy.

Pathologic evaluation

All pathological specimens were reviewed blindly to assess the post-operative pathology and pathological response in both groups according to Chevallier classification and residual cancer burden (RCB). Macroscopic and microscopic analyses were performed to establish the Chevallier classification which includes four grades, grade 1 is the disappearance of all tumor on microscopic and macroscopic levels, grade 2 is the presence of DCIS in breast only while lymph nodes are free, grade 3 is the presence of invasive carcinoma with stromal alteration, such as sclerosis or fibrosis and grade 4 is no or few modifications of the tumoral Appearance. The definition of pCR encompasses both class 1 and class2.

RCB score and class were determined using the MD Anderson Cancer Center's online calculator.[26] This system was developed to calculate RCB in breast in patients who received neoadjuvant treatment to predict distant relapse-free survival.[27] RCB system uses some variables including residual invasive tumor, average cellularity in the tumor bed, number of lymph nodes with metastases, and the size of the largest metastasis in the involved LNs, combined mathematically with a continuous index to define the four classes of RCB (RCB-0 through RCB-III). Although the system requires the use of a mathematical formula, a Web-based calculation script is present for free at MA-Anderson website to calculate the scores.[28]

Results

The 2 groups were well balanced as illustrated in table (1). Around 44.4% in group (A) and 46.9% in group (B) had breast cancer stage IIIB at presentation, which reflects the high risk in our treatment cohort.

Luminal subtype was the most prevalent subtype representing 92.6% (25 patients) in group A and 81.3% (26 patients) in group B.

All patients in both groups received four cycles of AC regimen. Eight patients (29.6%) in group (A) and nine patients (28.1%) in group (B) received additional twelve weeks of paclitaxel.

Pathological assessment post neoadjuvant treatment:

Pathological assessment of the patients after surgery showed that only four patients in group (A) (14.8%) and two patients (6.3%) in group B developed pCR and difference wasn`t statistically significant p=0.3. The difference between the two groups regarding other pathological responses in the T and N wasn't statistically significant as well. Final pathological stage illustrated in table (2)

When we applied the Chevallier system (table 4), our data showed that 4 (14.8%) patients in group (A) were grade 1, and two patients (6.3%) in group (B) were grade 1 (P-value 0.39), while 22 (81.5%) and 23 patients (71.9%) were grade 3 and in group (A) and (B) respectively (P value 0.38). In comparison, those with grade 4 which represents no change at all in the tumor were one patient (3.7%) and 7 patients (21.9%) in group (A) and (B) respectively with a p-value of 0.06, approaching significance in favor of the Metformin group "group A".

When we used RCB classification (table 3), we found that four patients (14.8%) and two patients (6.3%) in group (A) and (B) had CPR respectively with (P-value 0.39). Eleven patients (40.7%) in

group (A) had the worst RCB class "class 3" in comparison to 22 patients (68.8%) in group (B) and the difference was statistically significant in favor of the group A "Metformin group" (p=0.031) figure (3). The RCB index mean value in group (A) was 2.7, while in group (B) was 3.3 with (P-value 0.053), also approaching significance in favor of the Metformin group. Moreover, when the patients were stratified by their molecular subtypes, we didn't find a significant correlation between both groups in either luminal or her2 enriched subtypes.

There was no significant correlation between both the type of received neoadjuvant chemotherapy and the initial clinical stage with the pathological stage at surgery in our sample.

In the whole sample, 42 patients received AC regimen without taxane; 17 (40.5%) of them had RCB classes 0, 1, and 2, while 25 of them (59.9%) had RCB class 3. Among the 17 patients who received taxane plus AC, nine patients (52.9%) had RCB classes 0, 1, and 2, and eight patients (47.1%) had RCB class 3 with (P-value of 0.38).

Safety and tolerability of Metformin with or without chemotherapy is demonstrated in "table (1) in the *supplementary materials*", showing that gastrointestinal adverse events like diarrhea, nausea, vomiting, and epigastric pain GI, II were slightly higher in group (A) and the difference was not statistically significance

Discussion

We did a prospective study to assess the effect of the addition of the oral hypoglycemic drug "Metformin" to conventional chemotherapy in the neoadjuvant settings using pathological response as the primary endpoint. In our study, the overall rate of pathological complete response was (10.16%). In the famous National Surgical Adjuvant Breast and Bowel Project (NSABP B27) trial the rate of pathological complete response was (13%) to those who received 4 cycles AC only

and (26%) to those who received 4 cycles AC followed by 4 cycles docetaxel[29]. In a large meta-analysis of 12 trials of neoadjuvant chemotherapy in breast "the Cortazar" meta-analysis[7] the rate of complete pathological response in all biological subtypes was (22%), (8-16%) for those with hormonal positive & HER2 negative tumor and (31%) for hormonal positive & HER2 positive tumors when trastuzumab was given with chemotherapy. The low rate of pathological complete response in our study may be attributed to the low rate of taxane use (28.8%), the prevalence of hormonal positive & HER2 negative subtype (57.6%) and the lake of trastuzumab in HER-2 positive patient who were (42.3%) of the sample.

The main finding of our study is that the rate of complete pathological response was 14.8% (4 patients) in group (A) "Metformin group" versus 6.3% (2 patients) in group (B) "non-metformin group" with a p-value of 0.39 which is comparable to what was published by Arce-Salinas et al in 2016 [30] where they carried out a randomized double-blinded trial included (48) hormonal positive & Her-2 negative non-diabetic breast cancer patients. Twenty-eight patients received neoadjuvant chemotherapy (Anthracyclin and Taxanes) with Metformin 850 mg BID and 20 patients in the control arm received neoadjuvant chemotherapy without Metformin. The rate of PCR was 26.9% in the Metformin group versus 5.9% in the non-Metformin group with a (P-value of 0.22) which is still not statistically significant. The rate of complete pathological response in the abovementioned study was numerically higher than that in our study as they used Taxanes in all patients and they did not include HER-2 positive subtype as we did.

The justification for including Her-2 positive patients is that Metformin has been shown to inhibit both the tyrosine kinase activity and the expression of the human epidermal growth factor receptor 2 (HER2) in vitro models of HER2-positive breast cancer cells[31-34]. Metformin treatment cause lowering of circulating levels of insulin and insulin-like growth factor (IGF-I), and to cell-

autonomous suppression of the" mTOR" pathway. Thus Metformin not only directly targets HER-2 receptor but also target central mechanisms implicated in refractoriness to HER-2 targeted therapy such as IGF-1 and mTOR pathway[35]. Begona Martin-Castillo et al published their phase 2 trial of neoadjuvant Metformin in combination with Trastuzumab and chemotherapy in women with early HER-2 positive breast cancer "the METTEN study"[35] which included 58 HER-2 positive non-metastatic breast cancer patients randomized to group (A) 29 patients who received 12 cycles of weekly Taxol plus Trastuzumab followed by 3-weekly FE75C plus Trastuzumab with Metformin 850 mg BID from the start of chemotherapy or equivalent regimen without Metformin as group (B). The pathological complete response was 65.5% (19 of 29 patients) in group (A) and 58.6% (17 of 29 patients) in group (B) with p-value of 0.589 which still also not statistically significant. Unfortunately, non-of the 25 HER-2 positive patients in our study in group (A & B) received Trastuzumab as it is not covered by the state in the neoadjuvant settings and none of our patients could afford the expense of buying it.

Data from "Asian Breast Cancer Database" showed that the diabetic patients who were receiving Metformin when breast cancer was diagnosed showed a better prognosis only if they had hormonal receptor-positive with HER-2 receptor positive tumors[36]. Another important note, in an analysis of ALLTO which is phase III trial of HER-2 directed therapy in HER-2 positive patients where they randomized patients to receive 1 year of Trastuzumab alone, Lapatinib alone, their sequence or their combination, they found that diabetic patients who were receiving Metformin had a statistically significant beneficial effect when their primary tumor was hormonal positive & HER-2 positive[37]. It is well known that Metformin causes a reduction of (Ki-67) level, especially in (HER2+ve) tumors.[38] Moreover, Andrea Decensi et al found in their randomized pre-surgical trial of the effect of Metformin on breast ductal carcinoma in situ proliferation in comparison to a

placebo, Metformin causes a 40% reduction of KI-67 level in the Metformin arm in (HER2-+ve) DCIS particularly when they co-express estrogen receptor[23]. In our study in the Metformin group patients with HR+ & HER-2+ve tumor had numerically better pathological response. Six patients (85.7%) had RCB classes 0, 1, and 2 versus only one patient (14,3%) among this group had RCB class 3 with a p-value of 0.18 which wasn`t statistically significant which may be due to the small number of our sample. In group (B) control group patients with the same tumor subtype (HR+ & HER-2+) didn`t show better pathological response as present group (A) (4 patients (40%) had RCB classes 0, 1, and 2 Vs 6 patients (60%) had RCB class3). These results may confirm what was observed in previous studies that Metformin may have a good effect on breast cancer tumors with HR+ & HER-2+ subtype and this observation warranted more prospective trials to confirm it.

When we used the RCB scoring system in the analysis of the pathological response and combined class (RCB 0/1/2) versus RCB class 3, we found that RCB class 3 was 40.7% in group (A) versus 68.8% in group (B) which was statistically significant with a p-value of 0.031. We used RCB class 0/1/2 vs 3 based on what was published by Campbell et al 2017[39] where they used residual cancer burden classes as a predictor of recurrence risk and found that RCB class 3 significantly predicts high recurrence risk after adjusting for age, clinical stage, and hormonal receptor status[39].

The maximum effective dose of Metformin used in patients with T2DM to treat hyperglycemia is 1000 mg twice daily. However, the Metformin dose that achieved the maximal anti-cancerous effect is unclear. Results from two xenograft models preclinical studies reported that the human equivalent of 1500–2250 mg/day was needed to inhibit tumorigenesis.[40-42] Kim et al in phase II randomized trial (METEOR) trial of neoadjuvant Metformin plus Letrazole versus placebo plus Letrazole (still an ongoing trial no results published yet) used the dose of 1000mg BID[43]. Whereas the ongoing NCIC CTG MA32 phase III clinical trial is testing the effect of adjuvant Metformin,

utilizing a dosage of 1700 mg/day. It is the most advanced adjuvant trial investigating the effect of Metformin versus placebo on invasive disease-free survival and other outcomes in 3,649 women[44]. Arce-Salinas et al.,[45] and Martin-Castello et al.,[46] as mentioned earlier used the same dose we utilized (1700mg/day).

Our study confirms that Metformin is a tolerable and safe addition to chemotherapy, the dropout rate in Metformin arm was (11.8%). Seven out of 59 patients in the Metformin arm withdrew because of Metformin-related gastrointestinal upset while in the METTEN study the dropout rate in the Metformin arm was 13% (5 out of 38 patients).

A major limitation in our study is the lack of using anti-her-2 drugs with neoadjuvant chemotherapy in Her-2 positive patients due to lack of resources.

Another limitation in our study is not using Ki67 testing before and after chemotherapy as Ki67 is a good marker to be used to see the effect of investigational new drugs in the neoadjuvant setting. Finally, the lack of correlation of pathological complete response to overall- survival.

Conclusion

Metformin can increase the rate of pathological response when combined with chemotherapy. We found that adding Metformin to neoadjuvant chemotherapy resulted in better pathologic response by using residual cancer burden score. Patients with hormone receptor and Her-2 positive tumors may have the most benefit of adding metformin but a larger trial is needed to confirm this finding.

Metformin is a safe addition to neoadjuvant chemotherapy in breast cancer patients and is well-tolerated.

Declarations

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Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose

Ethics approval

Informed consent was taken from all patients after approval from the ethics committee in our University "The Ethics Committee of the Faculty of Medicine-Alexandria University is constituted and operates according to ICH GCP guidelines and applicable local and institutional regulations and guidelines which govern EC operation

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Data Availability" statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author contributions:-

Shaimaa.M.Elkhayat: conceptualization, methodology, validation, investigation, resources, data curation, writing - original draft. **Mohamed Abouegylah**: writing - review & editing, supervision. **Dina Abdallah**: investigation, resources. **Ahmed Gaber Geweil**: supervision, project administration **Ashraf.M. Elenbaby:** supervision, project administration Omar Shebl Zahra: supervision, project administration

References

1. Taghian A, El-Ghamry M, Merajver SJU. Overview of the treatment of newly diagnosed, non-metastatic breast cancer 2016.

2. Tryfonidis K, Senkus E, Cardoso MJ, Cardoso FJNrCo. Management of locally advanced breast cancer—perspectives and future directions 2015;**12**: 147-62.

3. Ramani PA, Niharika VS, Lakhsmi BKM, Jahnavi S, Reddy GVSJISJ. Incidence of locally advanced breast cancer in women presenting to a tertiary care center 2019;**6**: 3626-31.

4. El Saghir NS, Adebamowo CA, Anderson BO, Carlson RW, Bird PA, Corbex M, Badwe RA, Bushnaq MA, Eniu A, Gralow JRJTB. Breast cancer management in low resource countries (LRCs): consensus statement from the Breast Health Global Initiative 2011;**20**: S3-S11.

5. Madigan LI, Dinh P, Graham JD. Neoadjuvant endocrine therapy in locally advanced estrogen or progesterone receptor-positive breast cancer: determining the optimal endocrine agent and treatment duration in postmenopausal women—a literature review and proposed guidelines. *Breast Cancer Research* 2020;**22**: 77.

6. Generali D, Corona SP, Pusztai L, Rouzier R, Allevi G, Aguggini S, Milani M, Strina C, Frati AJJocr, oncology c. Benefit of the addition of hormone therapy to neoadjuvant anthracycline-based chemotherapy for breast cancer: comparison of predicted and observed pCR 2018;**144**: 601-6.

7. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, Bonnefoi H, Cameron D, Gianni L, Valagussa PJTL. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis 2014;**384**: 164-72.

8. Larsson SC, Mantzoros CS, Wolk AJIjoc. Diabetes mellitus and risk of breast cancer: a meta-analysis 2007;**121**: 856-62.

9. Liao S, Li J, Wei W, Wang L, Zhang Y, Li J, Wang C, Sun SJAPJCP. Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature 2011;**12**: 1061-5.

10. Wolf I, Sadetzki S, Catane R, Karasik A, Kaufman BJTlo. Diabetes mellitus and breast cancer 2005;**6**: 103-11.

11. Renehan AG, Frystyk J, Flyvbjerg AJTiE, Metabolism. Obesity and cancer risk: the role of the insulin–IGF axis 2006;**17**: 328-36.

12. Goodwin PJ, Pritchard KI, Ennis M, Clemons M, Graham M, Fantus IGJCbc. Insulin-lowering effects of metformin in women with early breast cancer 2008;**8**: 501-5.

13. Pollak M. Insulin-like growth factor-related signaling and cancer development *Cancer Prevention*ed.: Springer, 2007: 49-53.

14. Denley A, Carroll JM, Brierley GV, Cosgrove L, Wallace J, Forbes B, Roberts CTJM, biology c. Differential activation of insulin receptor substrates 1 and 2 by insulin-like growth factor-activated insulin receptors 2007;**27**: 3569-77.

15. Dowling RJ, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. *BMC medicine* 2011;**9**: 33.

16. Liao S, Li J, Wei W, Wang L, Zhang Y, Li J, Wang C, Sun S. Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature. *Asian Pac J Cancer Prev* 2011;**12**: 1061-5.

17. Dowling RJ, Goodwin PJ, Stambolic VJBm. Understanding the benefit of metformin use in cancer treatment 2011;**9**: 33.

18. Dowling RJ, Niraula S, Chang MC, Done SJ, Ennis M, McCready DR, Leong WL, Escallon JM, Reedijk M, Goodwin PJJBCR. Changes in insulin receptor signaling underlie neoadjuvant metformin administration in breast cancer: a prospective window of opportunity neoadjuvant study 2015;**17**: 32.

19. Goodwin PJ, Ennis M, Pritchard KI, Trudeau ME, Koo J, Madarnas Y, Hartwick W, Hoffman B, Hood NJJoco. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study 2002;**20**: 42-51.

20. Pasanisi P, Berrino F, De Petris M, Venturelli E, Mastroianni A, Panico SJIjoc. Metabolic syndrome as a prognostic factor for breast cancer recurrences 2006;**119**: 236-8.

21. Mulligan AM, O'Malley FP, Ennis M, Fantus IG, Goodwin PJJBcr, treatment. Insulin receptor is an independent predictor of a favorable outcome in early stage breast cancer 2007;**106**: 39-47.

22. Gong J, Kelekar G, Shen J, Shen J, Kaur S, Mita MJTo. The expanding role of metformin in cancer: an update on antitumor mechanisms and clinical development 2016;**11**: 447-67.

23. DeCensi A, Puntoni M, Guerrieri-Gonzaga A, Cazzaniga M, Serrano D, Lazzeroni M, Vingiani A, Gentilini O, Petrera M, Viale GJCPR. Effect of metformin on breast ductal carcinoma in situ proliferation in a randomized presurgical trial 2015;**8**: 888-94.

24. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, Langer C, Murphy B, Cumberlin R, Coleman CN: CTCAE v3. 0: development of a comprehensive grading system for the adverse effects of cancer treatment *Seminars in radiation oncology* 2003; 13:176-81.

25. Therasse P, Eisenhauer E, Verweij JJEjoc. RECIST revisited: a review of validation studies on tumour assessment 2006;**42**: 1031-9.

26. Akbar S, Peikari M, Salama S, Nofech-Mozes S, Martel AL: Determining tumor cellularity in digital slides using resnet *Medical Imaging 2018: Digital Pathology* 2018; 10581:105810U.

27. Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, Assad L, Poniecka A, Hennessy B, Green MJJoCO. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy 2007;**25**: 4414-22.

28. Sahoo S, Lester SCJAop, medicine l. Pathology of breast carcinomas after neoadjuvant chemotherapy: an overview with recommendations on specimen processing and reporting 2009;**133**: 633-42.

29. Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahlenberg MS, Robidoux A, Margolese RG, Hoehn JL, Vogel VG, Dakhil SRJJoCO. Preoperative chemotherapy: updates of national surgical adjuvant breast and bowel project protocols B-18 and B-27 2008;**26**: 778-85.

30. Arce-Salinas C, Alamilla G, Flores-Diaz D, Deneken CZ, Mendoza-Galindo L, Ramirez-Morales R, Shaw-Dulin R, Santamaria-Galicia J, Alvarado-Miranda A, Villarreal-Garza C. Randomized, double blind trial to evaluate the safety and efficacy of metformin vs placebo plus neoadjuvant chemotherapy in locally advanced breast cancer: American Society of Clinical Oncology, 2016.

31. Kim J, Lee J, Kim C, Choi J, Kim AJTB. Anti-cancer effect of metformin by suppressing signaling pathway of HER2 and HER3 in tamoxifen-resistant breast cancer cells 2016;**37**: 5811-9.

32. Vazquez-Martin A, Oliveras-Ferraros C, Menendez JAJCc. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells 2009;**8**: 88-96.

33. Vázquez-Martín A, Oliveras-Ferraros C, del Barco S, Martín-Castillo B, Menéndez JAJC, Oncology T. mTOR inhibitors and the anti-diabetic biguanide metformin: new insights into the molecular management of breast cancer resistance to the HER2 tyrosine kinase inhibitor lapatinib (Tykerb®) 2009;**11**: 455-9.

34. Vazquez-Martin A, Oliveras-Ferraros C, Del Barco S, Martin-Castillo B, Menendez JJAoO. The antidiabetic drug metformin: a pharmaceutical AMPK activator to overcome breast cancer resistance to HER2 inhibitors while decreasing risk of cardiomyopathy 2009;**20**: 592-5.

35. Martin-Castillo B, Pernas S, Dorca J, Álvarez I, Martínez S, Pérez-Garcia JM, Batista-López N, Rodríguez-Sánchez CA, Amillano K, Domínguez SJO. A phase 2 trial of neoadjuvant metformin in combination with trastuzumab and chemotherapy in women with early HER2-positive breast cancer: the METTEN study 2018;**9**: 35687.

36. Kim HJ, Kwon H, Lee JW, Kim HJ, Lee SB, Park HS, Sohn G, Lee Y, Koh BS, Yu JHJBCR. Metformin increases survival in hormone receptor-positive, HER2-positive breast cancer patients with diabetes 2015;**17**: 64.

37. Sonnenblick A, Agbor-Tarh D, Bradbury I, Di Cosimo S, Azim Jr HA, Fumagalli D, Sarp S, Wolff AC, Andersson M, Kroep JJJoCO. Impact of diabetes, insulin, and metformin use on the outcome of patients with human epidermal growth factor receptor 2–positive primary breast cancer: Analysis from the ALTTO phase III randomized trial 2017;**35**: 1421.

38. DeCensi A, Puntoni M, Gandini S, Guerrieri-Gonzaga A, Johansson HA, Cazzaniga M, Pruneri G, Serrano D, Schwab M, Hofmann UJBcr, treatment. Differential effects of metformin on breast cancer proliferation according to markers of insulin resistance and tumor subtype in a randomized presurgical trial 2014;**148**: 81-90.

39. Campbell JI, Yau C, Krass P, Moore D, Carey LA, Au A, Chhieng D, Giri D, Livasy C, Mies CJBcr, treatment. Comparison of residual cancer burden, American Joint Committee on Cancer staging and pathologic complete response in breast cancer after neoadjuvant chemotherapy: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657) 2017;**165**: 181-91.

40. Huang X, Wullschleger S, Shpiro N, McGuire VA, Sakamoto K, Woods YL, Mcburnie W, Fleming S, Alessi DRJBJ. Important role of the LKB1–AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice 2008;**412**: 211-21.

41. Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, Zabezhinski MA, Kovalenko IG, Poroshina TE, Semenchenko AV, Provinciali MJEg. Effect of metformin on life span and on the development of spontaneous mammary tumors in HER-2/neu transgenic mice 2005;**40**: 685-93.

42. Anisimov V, Egormin P, Bershtein L, Zabezhinskii M, Piskunova T, Popovich I, Semenchenko AJBoeb, medicine. Metformin decelerates aging and development of mammary tumors in HER-2/neu transgenic mice 2005;**139**: 721-3.

43. Kim J, Han W, Kim E-K, Jung Y, Kim H-A, Chae SM, Lee ES, Ahn S-H, Kim TH, Jeong J. Phase II randomized study of neoadjuvant metformin plus letrozole versus placebo plus letrozole for ER-positive postmenopausal breast cancer [METEOR Study]: American Society of Clinical Oncology, 2019.

44. Camacho L, Dasgupta A, Jiralerspong S. Metformin in breast cancer-an evolving mystery: BioMed Central, 2015.

45. Rubovszky G, Horváth Z. Recent Advances in the Neoadjuvant Treatment of Breast Cancer. *J Breast Cancer* 2017;**20**: 119-31.

46. Martin-Castillo B, Pernas S, Dorca J, Álvarez I, Martínez S, Pérez-Garcia JM, Batista-López N, Rodríguez-Sánchez CA, Amillano K, Domínguez S, Luque M, Stradella A, et al. A phase 2 trial of neoadjuvant metformin in combination with trastuzumab and chemotherapy in women with early HER2-positive breast cancer: the METTEN study 2018;**9**.

Figures captions:-

Fig 1. A diagram shows the included and excluded patients in the study.

Fig 2. Pathological response by residual cancer burden classes. 40.7% in group (A) had RCB class "class 3" in comparison to (68.8%) in group (B) and the difference was statistically significant in favor of the metformin group (p=0.031).

Tables

Table1: Baseline clinic-pathological criteria of the two study groups

	A	**B**	**P**
Mean age	49	47	0.83
BMI	32.8	33.9	0.58
Menopausal status:			0.43
Pre	Pre 15	Pre 21	
post	Post 12	Post 11	
Side			0.2
Right	Right 18	16	
Left	Left 9	16	
Stage			0.58
IIA	7	11	
IIIA	8	6	
IIIB	12	15	
Pathology			0.86
IDC	25	30	
ILC	2	2	
ER status			0.74
Positive	23	26	
Negative	4	6	
Her2			0.2
Positive	9	16	
Negative	18	16	
Grade			
I	1	0	0.57
II	23	27	
III	3	5	

Table 2: Pathological response to chemotherapy

Pathological characteristics	Group(A) (n = 27)		Group (B) (n = 32)		P
	No.	**%**	**No.**	**%**	
T stage					
T0	4	14.8	2	6.3	^{MC}p= 0.263
T1	9	33.3	7	21.9	
T2	9	33.3	17	53.1	
T3	4	14.8	2	6.3	
T4	1	3.7	4	12.5	
LN					
N0	9	33.3	6	18.8	^{MC}p= 0.331
N1	10	37.0	9	28.1	
N2	6	22.2	12	37.5	
N3	2	7.4	5	15.6	
Stage					
CPR	4	14.8	2	6.3	^{FE}p=0.398
IA	7	25.9	6	18.8	0.508
IB	10	37.0	12	37.5	0.971
IIA	1	3.7	1	3.1	^{FE}p=1.000
IIB	0	0.0	1	3.1	^{FE}p=1.000
IIIA	3	11.1	5	15.6	^{FE}p=0.715
IIIB	2	7.4	5	15.6	^{FE}p=0.437
CPR, stage I,II	22	81.5	22	68.8	0.263
Stage III	5	18.5	10	31.3	

Table 3: Comparison between the two studied groups according to residual cancer burden classification

RCB classification	Group (A) (n = 27)		Group (B) (n = 32)		Test of sig.	P
	No.	**%**	**No.**	**%**		
RCB class						
CPR	4	14.8	2	6.3	χ^2=1.176	^{FE}p=0.398
Class 1	0	0.0	1	3.1	χ^2=0.858	^{FE}p=1.000
Class 2	12	44.4	7	21.9	χ^2=3.417	0.065
Class 3	11	40.7	22	68.8	χ^2=4.661*	0.031*
CPR & Class 1 & 2	16	59.3	10	31.3	χ^2=4.661*	0.031*
Class 3	11	40.7	22	68.8		
RCB index value						
Min. – Max.	0.0 – 4.55		0.0 – 4.50		U= 305.0	0.053
Mean ± SD	2.71 ± 1.41		3.30 ± 1.22			
Median	3.03		3.76			

Table 4: Comparison between the two studied groups according to Chevallier classification

Chevallier classification	Group (A) (n = 27)		Group (B) (n = 32)		Test of sig.	p
	No.	**%**	**No.**	**%**		
Chevallier						
Grade 1	4	14.8	2	6.3	χ^2=1.176	^{FE}p=0.398
Grade 3	22	81.5	23	71.9	χ^2=0.747	0.388
Grade 4	1	3.7	7	21.9	χ^2=4.126	^{FE}p=0.060

Grade 1: Disappearance of all tumor either on macroscopic or microscopic assessment
Grade 2: Presence of in situ carcinoma in the breast and no invasive tumor in breast or lymph nodes
Grade 3: Presence of invasive carcinoma with stromal alteration, such as sclerosis or fibrosis
Grade 4: No or few modifications of the tumoral Appearance

Figures

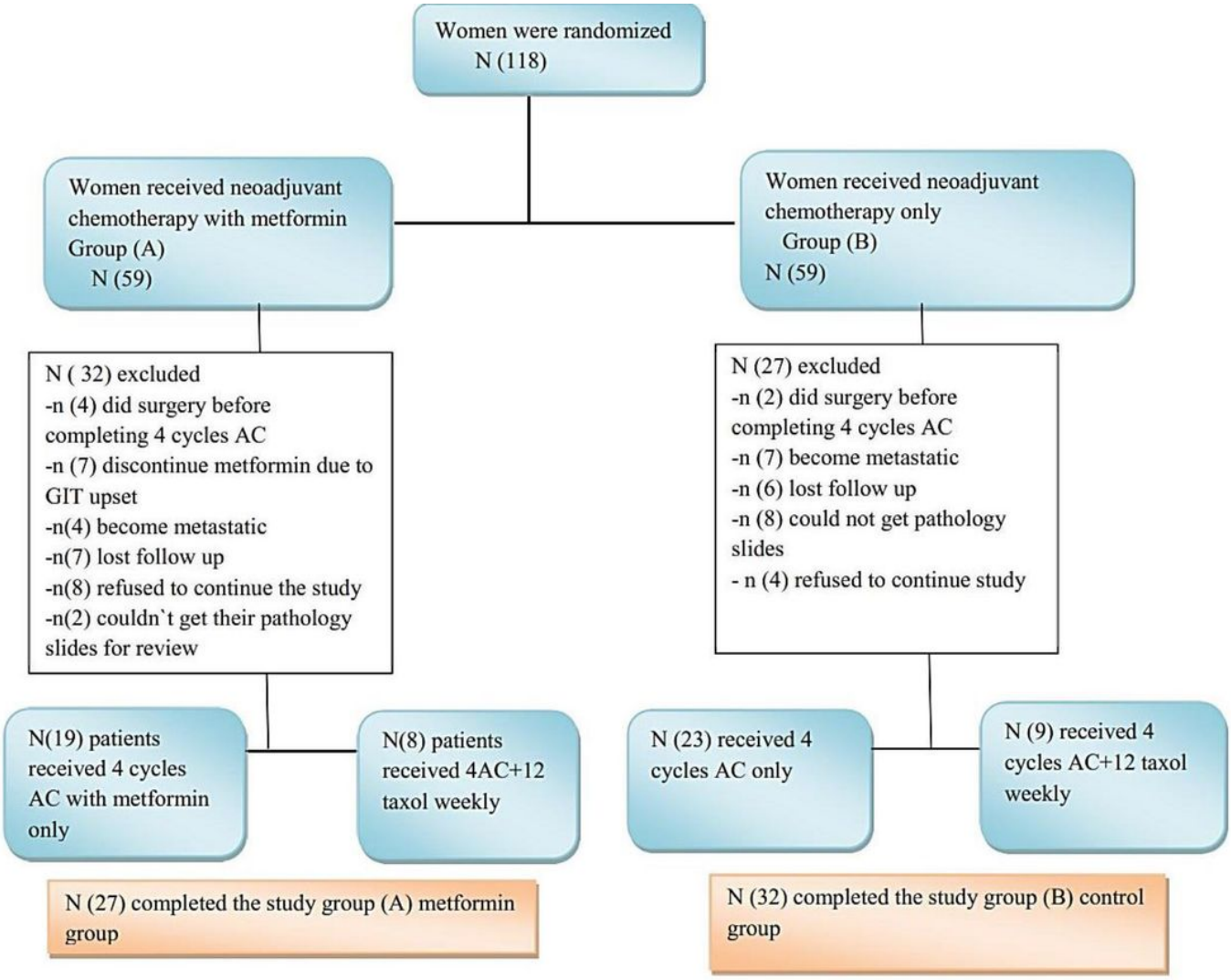

Fig 1.

Figure 1

A diagram shows the included and excluded patients in the study.

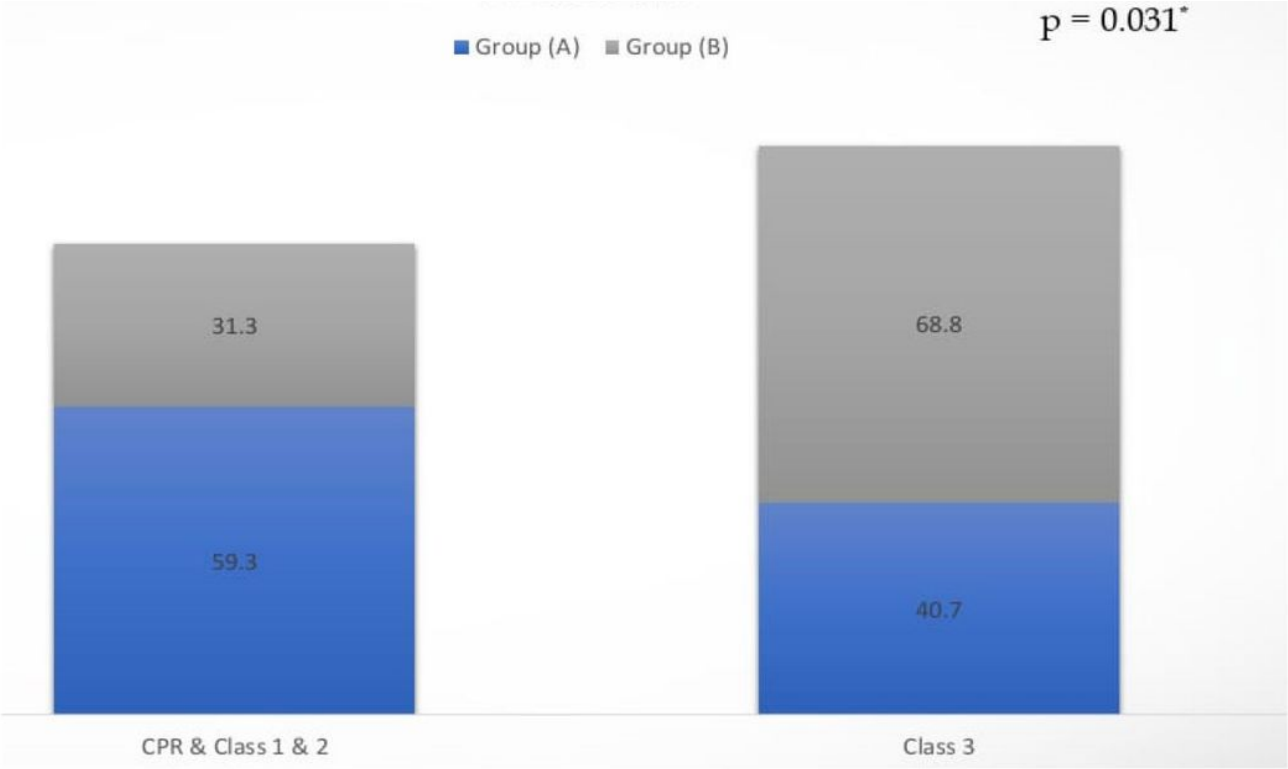

Figure 2

Figure 2

Pathological response by residual cancer burden classes. 40.7% in group (A) had RCB class "class 3" in comparison to (68.8%) in group (B) and the difference was statistically significant in favor of the metformin group (p=0.031).

Supplementary Files

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