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Prediction of response to neoadjuvant therapy in triple negative breast cancer

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Abstract

Triple-negative breast cancer (TNBC) is characterized by heterogeneity of molecular gene expression, aggressive clinical course, higher metastatatic rate, and shorter survival. Neoadjuvant chemotherapy (NAC) produces high response allowing down staging and making breast-conserving surgery (BCS) possible. Pathologic complete response (pCR) is used as an important endpoint in the treatment of TNBC. There is an unmet need to predict which subgroup of TNBC will get benefit from NAC. The exact subtype of TNBC using immunohistochemistry (IHC) in addition to metabolic imaging using FDG-PET are among the important suggested predictive factors. The aim of this prospective study is to investigate the value of metabolic tumor parameters (assessed at baseline with FDG-PET) and immuno-histochemical markers in the early prediction of pCR and survival in non-metastatic TNBC. A prospective study including 50 adult patients (age ≥ 18 years old) with non-metastatic TNBC with disease stage I to III who received NAC at clinical oncology department of Cairo University and medical oncology department at El-Tadamun hospital in Port Said in the period from December 2016 to December 2019.PET/CT was done baseline and after NAC. Biomarkers were done on the initial pathology including TIL, CK56, EGFR and AR1. Analysis of pCR with OAS and PFS was done. The mean age was 49 years old, premenopausal patients represented 64%, cases with T2 were 62%, metastatic axillary lymph nodes were found in 70% most of them were N1. As regard biomarkers of TNBC, there was predominance of TILs (56%) and CK5/6 (62%), while EGFR and AR1 presented a minority (22% each). The pCR was 78%. Overall survival (OAS) was significantly affected by tumor size (p = 0.03), high CK5/6 (p = 0.001), and pCR (p = 0.003) while TILs, EGFR and AR1 were insignificant (p = 0.89, 0.07, 0.262 respectively). PET/CT results correlates with pCR (p =0.001), OAS (p =0.001), and PFS (p =0.001). OAS was significantly affected by tumor size (T) and high CK5/6 pCR and response on PET/CT.

Keywords: TNBC, PET/CT, pCR, NAC, biomarkers, breast cancer.

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1. Introduction

Triple-negative breast cancer (TNBC), characterized by absence of expression of estrogen and progesterone receptors (ER, PR), and human epidermal growth factor receptor 2 (HER2), has been shown to be molecularly heterogeneous in gene expression analyses [1-2]. The majority of TNBC patients have aggressive clinical course, higher rate of metastases, and shorter survival, thus requiring aggressive multidisciplinary treatment with surgery, radiation and chemotherapy [3-5]. The remaining TNBCs are less aggressive, have a more favorable prognosis, and may benefit from certain hormonal or targeted therapies. Although neoadjuvant chemotherapy (NAC) does not improve survival when compared with adjuvant chemotherapy, it is increasingly used in operable breast cancer to downstage the breast tumor and to make breast-conserving surgery possible [6-8]. The TNBC subtype is characterized by its higher aggressiveness and poorer outcome compared with other subtypes but also by its high responsiveness to NAC, called the "triple-negative paradox" [9-13]. Indeed, a pathologic complete response (pCR) is often reached at the end of NAC and is associated with a more favorable long-term outcome. In contrast, women who do not achieve pCR have a higher risk of relapse and reduced overall survival [14-16]. This explains why pCR is often used as an important endpoint in the treatment of TNBC. One other important characteristic of TNBC is the diversity within this subgroup, as it includes distinct molecular subtypes. There is a large unmet need to predict which subgroup of TNBC is more likely to benefit from neoadjuvant chemotherapy in a step towards better tailoring of the neoadjuvant therapy. Among the important suggested predictive factors comes the exact subtyping of TNBC using immunohistochemistry in addition to metabolic imaging using FDG-PET.

2. Patient and methods

A prospective study including 50 female patients with triple negative breast cancer TNBC. Non metastatic patients eligible for neoadjuvant chemotherapy will be included.

All pathologically proven TNBC stage I to III, age 18 years. All patients were with adequate hepatic, renal, and bone marrow function, not pregnant or nursing, fertile patients must use effective contraception, and no prior chemotherapy or endocrine therapy. Chemotherapy was one of two regimens. Regimen I: Three cycles FEC/FAC every 21 days then, 3 cycles Docetaxel every 21 days (5FU 500mg/m^2, Adriamycin 50mg /m^2 Epirubcin 100 mg/m^2, Cyclophosphamide 500mg/m^2, Docetaxel 75mg/m^2). Regimen II: Four cycles AC every 21 days then, 4 cycles (12 weeks) paclitaxel (Adriamycin 60mg/m^2, cyclophosphamide 600mg /m^2, Paclitaxel 80mg/m^2 (weekly for 12 weeks). Pathological biomarkers including EGFR, CK 5/6, androgen receptors (AR), tumor infiltrating lymphocytes (TILS) was done. Patients underwent Surgery either modified radical mastectomy (MRM) or conservative mastectomy (CBS) and axillary evacuation with assessment of the pCR after neoadjuvant chemotherapy. Pathological complete remission (pCR) was correlated with Pet/CT and biomarkers. Analysis between disease free survival (DFS) and response to treatment by Pet/CT, response to treatment by pCR, and response to treatment by biomarkers. Multivariate analysis of response to treatment by clinicoepidemiological data, pet/scam, biomarkers data and DFS, and Overall survival (OAS).

2.1. Statistical analysis

All data tabulated and statistically studied by descriptive analysis as well as survival analysis in relation to different clinico-epidemiological factors. Correlation of the biomarkers with pCR done by Chi square test. Survival defined as a time from presentation to death or date of last follow up. Disease-free survival defined as the time from achieving CR to relapse, death or last follow up. Survival analysis was performed using Caplan Meier method for both overall survival and disease-free survival. Univariate and multivariate analysis using COX regression module performed to test the power of relation between the independent variables and overall survival as well as disease free survival. A probability value (P-value) less than 0.05 considered significant. The statistical calculations done using computer programs Microsoft Excel version 7 and SPSS (statistical package for the social science) statistical programs.

3. Results and discussion

This is a prospective study including 50 patients with non-metastatic triple negative breast cancer (TNBC), received neoadjuvant chemotherapy in the period from December 2016 to December 2019 at clinical oncology department of Cairo University and medical oncology department at El-Tadamun hospital in Port Said with the follow up median 48.5 months (Range: 13-74), mean 47.5 ± 14.31. Patient clinic-epidemiological characters are described at table 1. Most of our cases were pre-menopausal 54% (N: 27 cases), with early T- stage 84% (N: 42 cases) and positive nodal status 70% (N: 35 cases). The breast mass size of the studied patients, it was founded that the mean breast mass size was (5.14 ± 0.942), ranged from 3 to 7 Cm. The median age was 47.5 months (Range: 29-75), mean 49.76 ± 12.45 with more than half 54% of patients were below 50 years old. The age distribution in our study sample is presented in Figure 1. There was correlation between pCR and complete response to treatment assessed by PET/CT both represented 78% of our cases. Pathological biomarkers were studied in our study with EGFR and AR1 were expressed in 22% of cases (11/50) while CK5/6 was positive in 62% of cases (31/50). The most common treatment regimen during the study was 4 cycles AC followed by 12 weeks taxol (60%, 30/50). Other regimen included FAC/docetaxel or FEC/docetaxel (20% each, 10/50). Surgery was mainly Conservative breast surgery (76%, 38/50) with only 24% undergoing modified radical mastectomy (MRM). Post operative radiotherapy was received in the majority of patients. (96%, 48/50). The univariate analysis of survival prognostic factors revealed that tumor size, CK5/6 expression, cPR, and complete response with PET/CT as prognostic factors of TNBC OAS (p = 0.03, <0.001, <0.001, 0.001 respectively) as in table 2. While with multivariate analysis there is no independent prognostic factor of OAS as in Table 3. OAS median 60 months (Range: 17-84), mean 56.68 ± 14.75. The 5-years OAS was 58%. Figures 1 showed the difference in OAS Kaplan Meier survival curves as regard tumor size, CK5/6, pCR, and PET/CT treatment assessment. he 2-years PFS was 96 %. PFS median 49 months (Range: 13-76), mean 48.4 ± 14.75 as shown in Figure 2. The univariate analysis of PFS revealed that pCR and treatment response assessment with PET/CT as the significant prognostic factors (p = 0.007, <0.001 respectively) as in table 5. Their effect on survival difference were shown at figures 9 and 10. The multivariate analysis showed that the PET/CT treatment response assessment as the most independent PFS prognostic factor as in table 4 (p = 0.005). We analyzed the relation between PET/CT treatment response and pathological biomarkers and pCR as in table 5. We found that there was statisticaly significant of CK5/6 and PET/CT treatment response as regard pCR (p = 0.003, <0.001 respectively). Among the various breast cancer subtypes, the TNBC subtype remains a challenge as it has a poor prognosis and as no specific targeted therapy is currently available [17]. Indeed, TNBC is associated with higher risk of distant recurrence and death, especially within the first 3 years after diagnosis. It is thus extremely important to identify the clinicobiologic, molecular, or imaging biomarkers that can early predict which TNBC tumors will respond to NAC. The ultimate goal is to better tailor neoadjuvant treatment in poorly responding TNBC [18-19]. There is a large unmet need to predict which subgroup of TNBC is more likely to benefit from NAC chemotherapy. Investigating the value of metabolic tumor parameters, and immunohistochemical markers in the early prediction of pCR in non-metastatic TNBC may be a prognostic factor. The present study, PET/CT results correlates with pCR (p =0.001), OAS (p =0.001), and PFS (p =0.001). CK 5/6 is the only biomarker that correlated with pathological complete response (pCR). OAS was significantly affected by tumor size (p = 0.03), high CK5/6 (p = 0.001), and pCR (p = 0.003). PFS was significantly affected by PET/CT treatment response assessment only on multivariate analysis (p= 0.005). The mean age was 49.76 ± 12.447 years (29 – 75). About more than half (54%) of them were < 50 years old. Most of the cases were premenopausal (64%), T2 (62%), N+ve (70%). The biomarkers were high in TILs (56%) and CK5/6 in (62%) while EGFR and AR1 represented a minority in the study population (22% each).

OAS was significantly affected by tumor size (p = 0.03), high CK5/6 (p = 0.001), and pCR (p = 0.003). PET/CT results correlates with pCR (p =0.001), OAS (p =0.001), and PFS (p =0.001). Our study confirms that the benefit of using the PET-guided treatment strategies to evaluate the benefit of neoadjuvant treatment and detection of early therapeutic changes in poorly responding women. In the studied sample, the mean age was 49.76 ± 12.447 years (29 – 75). About more than half (54%) of them were premenopausal and < 50 years old. This is similar to Pineda et al., (2019) study with the patients median age 47.88 (27.19–78.92) mostly premenopausal [20]. In Wu et al. (2019) study, the majority of patients age was also < 50 years old [21]. In contrast to other studies the age of patients was above 50 years [22-24]. This is in concordance with Yue et al., (2016) study, more than half of patients mean breast mass size was < 20mm [25]. And, in contrast to Groheux et al., (2016) study in which the median tumor size was 50 mm ranged from (18–170) mm [26]. In our study, the patients were mostly with T2 62%, N1 62%. This coincides with Wang et al., (2019) T2 was the most frequent (68.5%) of patients, while N0 and N1 were founded to be equally presented (41%) [27]. Similarly, in Loibl et al. (2018), T2 was 72% whereas In Nemeth et al. (2021) the majority of patients was T2, and N0 tumors [28-29]. In Wei et al., (2021), the majority of patients had low clinical tumor stage (< 3) and nodal stage (< 1) [30]. In the present study, about more than half of the patients (56%) were founded to have high levels of tumor infiltrating lymphocytes (TILs), there was no statistically significant difference between the two groups of patients (according to TILs presence) regarding their OAS and PFS (p = 0.89, 0.86 respectively). Also, TILs did not show correlation with pCR (p= 0.206). This may be due to the small sample size and may be the low intratumoral stoma which was not measured. This contradictory to Lee et al., (2020) study showing a significant association between the percentage of TILs and positive response to combination therapy with adriamycin, cyclophosphamide, and docetaxel was identified [31]. Also, Sawasdee et al., (2022) reported an enhancing chemotherapeutic response via tumor cell sensitization to T-cell mediated cytotoxicity [32]. In addition to the effect of the presence of TILs, there is a role of the location of TILs; as in Kim et al., (2021) study, pCR rate was significantly higher when the lymphocytic infiltration to the intratumoral stroma outnumbered that to the peritumoral stromal area [33]. As, TILs can be grouped into intratumoural (i.e. those with direct contact to tumour cells), stromal (i.e. those between the tumour cells) and LPBC (lymphocyte predominant BC, i.e. if there are more lymphocytes than tumour cells). Lymphocyte infiltration used as a continuous factor is predictive of neoadjuvant chemotherapy response, and tumour specimens classified as LPBC had significantly increased pCR rates compared to non-LPBC [34]. Moreover, an independent association between TILs and higher responses to trastuzumab and chemotherapy was confirmed in primary HER2+ disease, and an underlying correlation between TILs and immune genes has been found. TILs have also shown to be indicative of good prognosis after chemotherapy, particularly in TNBC [35]. Our study results have revealed that that EGFR and AR1 were absent in most of the studied patients (78% each) while CK5/6 was positive in most of the studied patients (62%). Our results coincide with Yan et al., (2020) showing low EGFR in 55% of patients and negative AR1 in 79% of patients whereas CK5/6 was mostly positive (71%) [36]. However, EGFR was high in TNBC in Wang et al., (2021) presenting 94% while CK5/6 was 75 % [37]. In our study, most of the studied patients (78%) had complete treatment response. pCR was a significant prognostic factor of survival and recurrence risk of our TNBC cases (p <0.001, 0.007 as regard OAS and PFS respectively). The pCR was higher than other studies. This may be explained by the absence of EGFR in most of the studied population. In contrast to Groheux et al., (2016) study, only (37%) of patients achieved pCR and (63%) had residual disease [26]. pCR was more frequent in patients with high-grade tumors, with smaller tumors, without (or with limited) clinical lymph nodes, and with a low AJCC stage. Yee et al., 2020, suggested that pCR is more predictive of recurrence-free survival when the subtype is considered [38]. Also, Miglietta et al. (2021) pointed that HER2 + and TNBC are associated with higher pCR rates when compared to hormone-receptor (HR)+/HER2- [39]. However, O'Shea et al., (2021) shown still low pCR in TNBC [40]. Pathological complete response (pCR) was 31% in Pineda et al. (2019) where 911 TNBC patient received neoadjuvant anthracycline and taxane based chemotherapy [20]. In this study, there was statistically significant difference between the patients (according to T classification) regarding their OAS (P= 0.03) while there was no statistically significant difference between the patients according to T classification regarding their PFS or according to N classification regarding their survivals. However, in Groheux et al., (2016) study, there was statistically significant difference between both of T and N score regarding the patient's pathologic response [26]. In our study, there was no statistically significant difference between the two groups of patients according to; AR1, regarding their survival either OAS or PFS (p = 0.262, 0.262 respectively). There was also no statistically significance when we analyzed the impact of AR1 on pCR (p= 0.633). The AR marker is an independent prognostic marker for better disease-free and overall survival in general. Patients with an AR+ tumour had a significantly lower pCR rate than those with an AR– tumour [41]. In TNBC there was no difference in regard to pCR between androgen-receptor positive or negative patients in Jongen et al., (2019) study, but others found a difference in favour of higher pCR rate for TNBC patients who were also AR– compared to AR+ TNBC patients [42-43]. In our study, there was no statistically significant difference between the two groups of patients according to; EGFR presence regarding OAS and PFS (p= 0.07, and 0.443 respectively) but there was a significant difference in OAS according to CK5/6 (p<0.001). There is a significant statistically correlation between CK5/6 and pCR (p = 0.003). Contradictory to Jiang et al. (2020) study, the patients with low expression (e.g. EGFR<15 %) had better survival outcome than those with EGFR>15 %. And in Elzamly et al., (2018) study, in which negative EGFR status correlated with the achievement of pCR [44]. In accordance to our results, Ryu et al., (2020) and Wang et al., (2021) reported that EGFR immunoreactivity correlated significantly with worse prognosis in their TNBC patients [37,45].

Kahraman et al., (2018) reported that basal cytokeratins had significant prognostic values in their cohort of patients [46]. In our study, cases were assessed with FDG PET/CT as a basal metastatic work up before starting neoadjuvant chemotherapy and at the end before proceed for surgical excision and assess pCR. Our results confirm the significant correlation between PET/CT results and pCR ($p < 0.001$). Also, PET/CT response assessment can be used as prognostic factor of TNBC survivals either OAS or PFS (P=0.001, <0.001 respectively). Our results correlate with the results reported by Abd El-Gaid et al., (2022) who reported that the high correlation between PET/CT response assessment and pathological response [47]. In his study, PET/CT after neoadjuvant treatment as regard breast disease detected 91.7% of patients with pCR, 69% of those with residual disease and 88.3% of those with no response (P value <0.001). However, as regard axillary lymph node disease, PET/CT predicted 93.9% of those revealed pathologic response (P value <0.001).

Meta-analysis of 19 studies and 920 patients included confirmed that F-FDG PET/CT is a considerable potential tool for the early prediction of pCR in TNBC or HER2-positive breast cancers, the sensitivity and specificity of FDG PET/CT in predicting histopathological response were, respectively, 84 % (95 % CI 78 – 88 %) and 66 % (95 % CI 62 – 70 %) [26]. The strengths of our study include its prospective design, the approach presented in our study can be used to evaluate the prognosis of patients at diagnosis and help clinical decision- making with respect to selecting the appropriate therapies for individual patients, consistent timing of imaging to evaluate early predictability and baseline biomarker (EGFR, CK 5/6, androgen receptor (AR) and TILs) before starting chemotherapy. Also, assessment of the pCR after neoadjuvant chemotherapy and its correlation with other prognostic markers**.** However, our study had some limitations; First, the small sample size, therefore a large study with a validation cohort is warranted for future research. Second, we don't determine the function of TILs in different locations of infiltration.

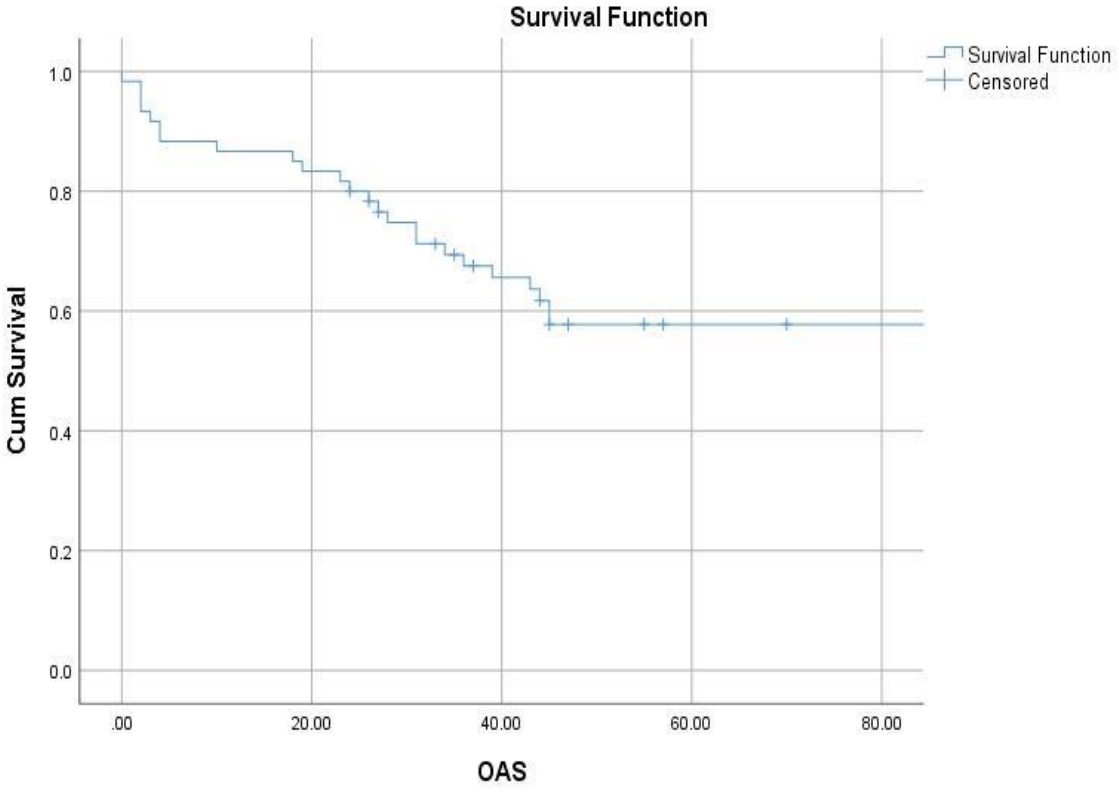

Figure 1 (a): Kaplan Meier curve represent OAS of TNBC.

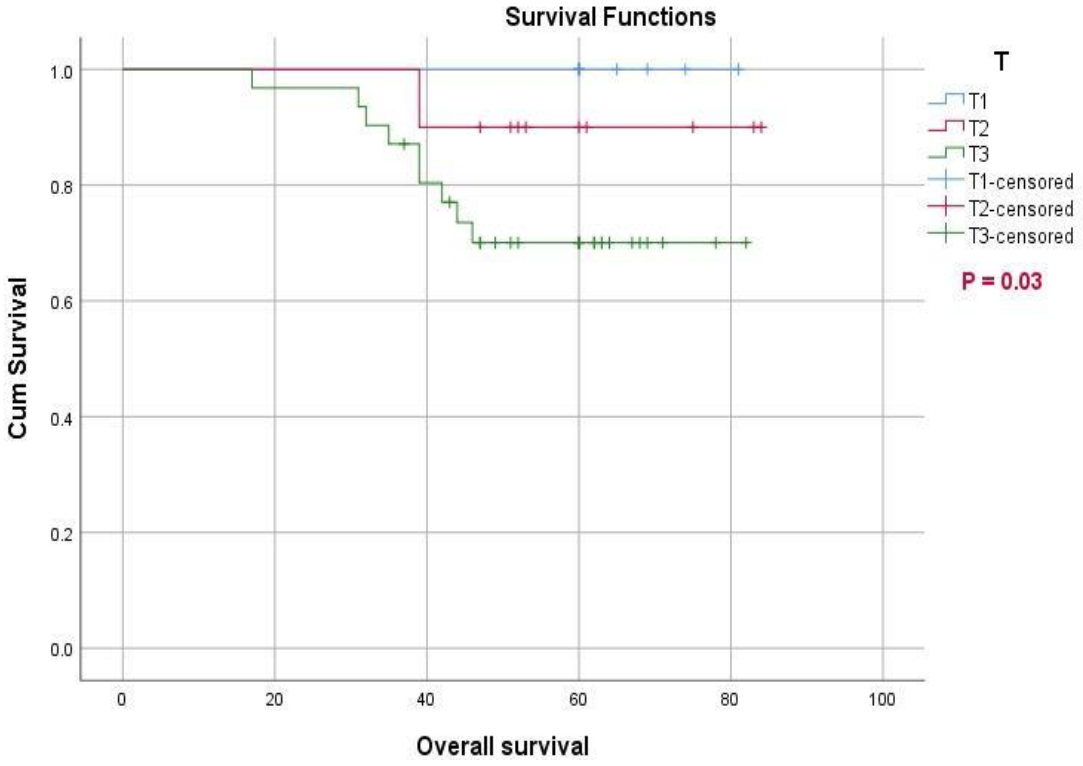

Figure 1 (b): The impact of tumor size (T) on OAS.

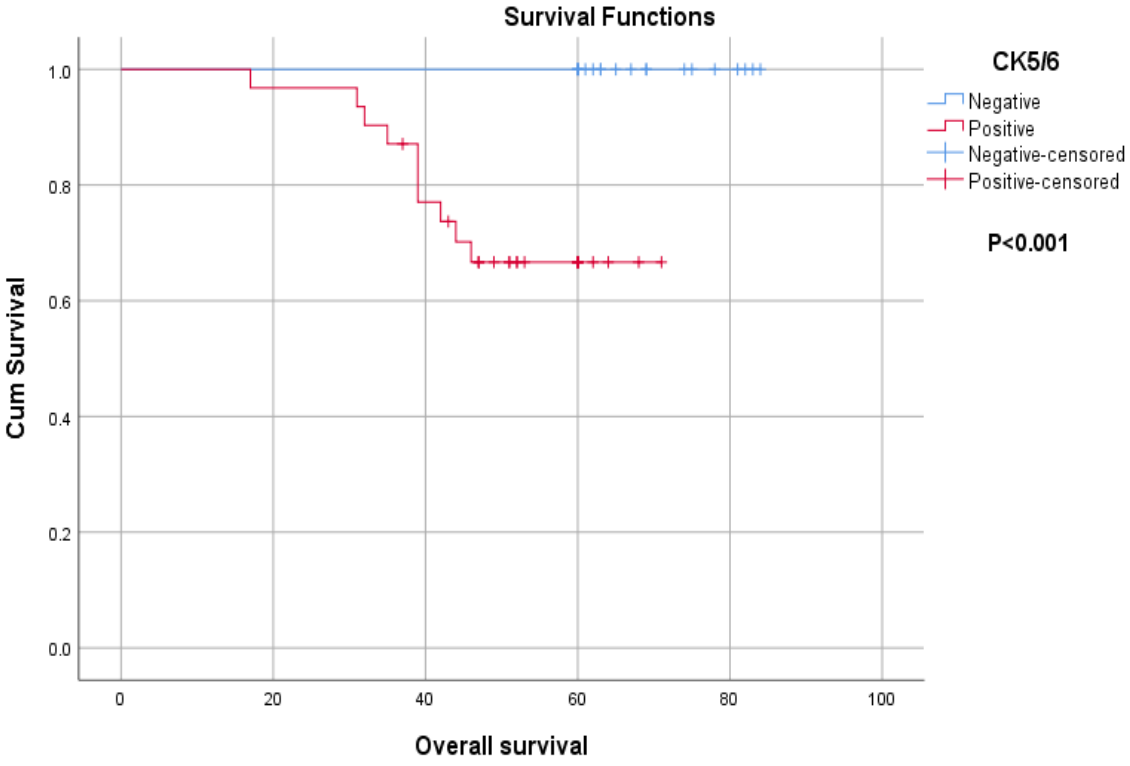

Figure 1 (c): The impact of CK5/6 on OAS.

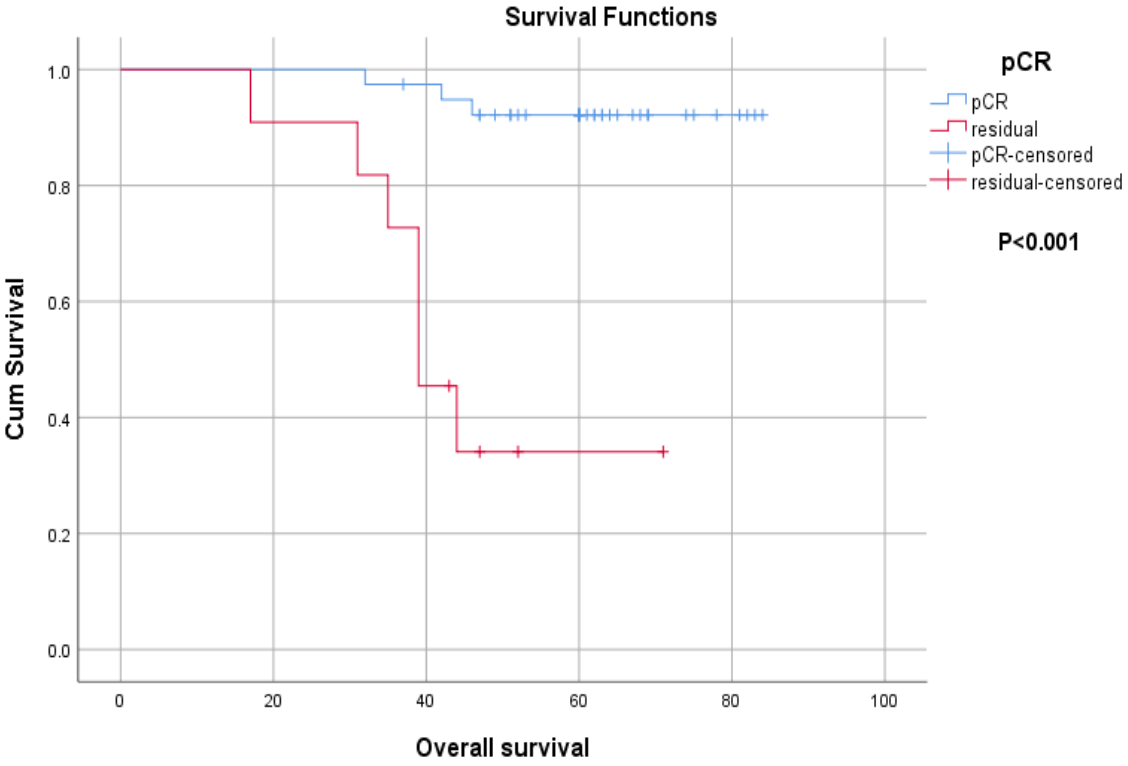

Figure 1 (d): The impact of pCR on OAS.

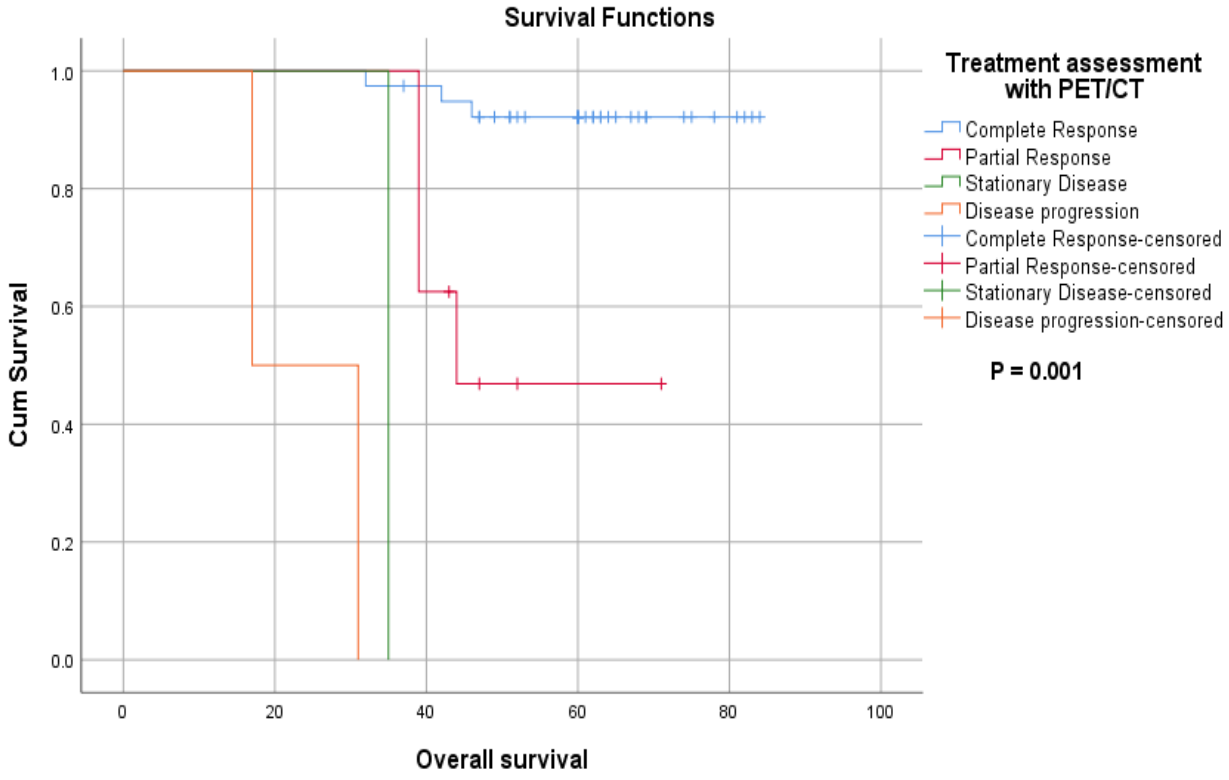

Figure 1 (e): OAS and PET/CT response assessment.

Table 1: Patient characteristics.

Characteristics	N:50	%
Age group		
< 50	27	54
≥ 50	23	46
Co-morbidities		
Diabetes mellitus	11	22
Hypertension	12	24
Ischemic heart disease	0	0
Hepatic disease	9	18
Renal disease	3	6
Menstrual status		
Premenopausal	27	54
Postmenopausal	23	46
Family history		
Yes	8	16
No	42	84
Tumor size		
T1	11	22
T2	31	62
T3	8	16
T4	0	0
Lymph node metastasis		
N0	15	30
N1	31	62
N2	4	8
TILS		
Low	22	44
High	28	56
EGFR		
Negative	39	78
Positive	11	22
AR1		
Negative	39	78
Positive	11	22
CK		
Negative	19	38
Positive	31	62
pCR		
pCR	39	78
Residual disease	11	22
PET/CT assessment		
Complete response	39	78
Partial response	8	16
Stationary disease	1	2
Progression	2	4

Table 2: Univariate analysis of prognostic factors of TNBC OAS.

Characteristics	95% CI	P-value
Age group		
< 50	0.894 (0.29 – 2.757)	0.845
≥ 50		
Co-morbidities		
Diabetes mellitus	0.835 (0.217 – 3.12)	0.793
Hypertension	1.61 (0.416 – 6.30)	0.485
Hepatic disease	0.885 (0.207 – 3.791)	0.87
Renal disease	1.48 (0.125 – 17.49)	0.754
Menstrual status		
Premenopausal	0.894 (0.290 – 2.757)	0.845
Postmenopausal		
Family history		
Yes	6.36 (0.719 -56.353)	0.065
No		
Tumor size		
T1	(0.103 – 0.115)	**0.030***
T2		
T3		
T4		
Lymph node metastasis		
N0	(0.128 – 0.142)	0.151
N1		
N2		
TILS		
Low	0.923 (0.297 – 2.865)	0.890
High		
EGFR		
Negative	4.27 (0.816 – 22.39)	0.070
Positive		
AR1		
Negative	2.286 (0.526 – 9.928)	0.262
Positive		
CK		
Negative	3.1(1.86 – 5.163)	**<0.001****
Positive		
pCR		
pCR	0.039 (0.004 – 0.344)	**<0.001***
Residual disease		
PET/CT assessment		
Complete response	(0.0001 – 0.001)	**0.001***
Partial response		
Stationary disease		
Progression		

Table 3: Univariate analysis of prognostic factors of TNBC PFS.

Characteristics	95% Confidence index	P-value
Age group		
< 50	0.846 (0.05 -14.329)	0.908
≥ 50		
Co-morbidities		
Diabetes mellitus	0.949 (0.882 – 1.02)	0.443
Hypertension	0.947 (0.879 – 1.02)	0.417
Hepatic disease	0.951 (0.88 – 1.019)	0.499
Renal disease	0.957 (0.901- 1.01)	0.715
Menstrual status		
Premenopausal	0.846 (0.05 -14.329)	0.908
Postmenopausal		
Family history		
Yes	0.171 (0.1 – 3.058)	0.181
No		
Tumor size		
T1	(0.711 – 0.729)	0.528
T2		
T3		
T4		
Lymph node metastasis		
N0	(0.147 – 0.161)	0.072
N1		
N2		
TILS		
Low	1.28 (0.076 - 21.78)	0.861
High		
EGFR		
Negative	0.949 (0.882 – 1.02)	0.443
Positive		
AR1		
Negative	0.949 (0.882 – 1.02)	0.443
Positive		
CK		
Negative	1.06 (0.975 – 1.172)	0.258
Positive		
pCR		
pCR	1.22 (0.925 – 1.61)	**0.007***
Residual disease		
PET/CT assessment		
Complete response	0 (0.0001 – 0.001)	**< 0.001***
Partial response		
Stationary disease		
Progression		

Table 4: The multivariate analysis of TNBC survival prognostic factors.

Factor	OAS			PFS		
	Hazard Ratio	95 % CI	P-value	Hazard Ratio	95 % CI	P - value
Family history	1	0.162-6170	1	0.290	0.016 – 5.376	0.406
Tumor size	1	0.352- 2.838	1	-	-	-
Lymph nodes	1	0.525- 1.904	1	0.451	0.190 – 1.070	0.071
EGFR	1	0.211 – 4.728	1	-	-	-
CK5/6	1	0.244 – 4.093	1	-	-	-
pCR	1	0.010 – 97.559	1	0.439	0.025 – 7.577	0.571
PET/CT assessement	1	0.025 – 40.006	1	14.36	2.282 – 90.436	0.005

Table 5: The correlation between pCR and PET/CT results and biomarkers.

Factor	pCR		P-value
	N	%	
PET/CT results			
Complete response	39	100	**<0.001 ****
Partial response	0	0	
Disease progression	0	0	
TILS			
Low	19	86.4	0.206
High	20	71.4	
EGFR			
Negative	29	74.4	0.242
Positive	10	90	
AR1			
Negative	19	79.5	0.633
Positive	8	72.7	
CK 5/6			
Negative	19	100	**0.003****
Positive	20	64.5	

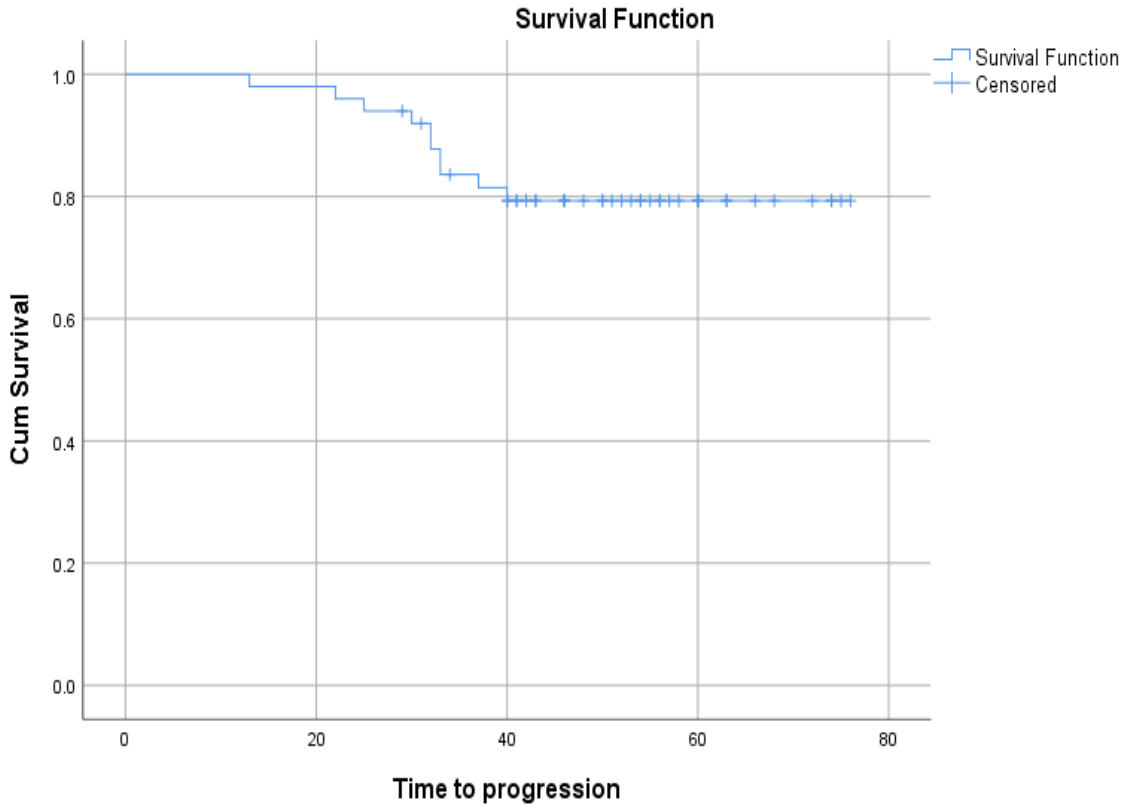

Figure 2 (a): Kaplan Meier curve representing PFS of TNBC.

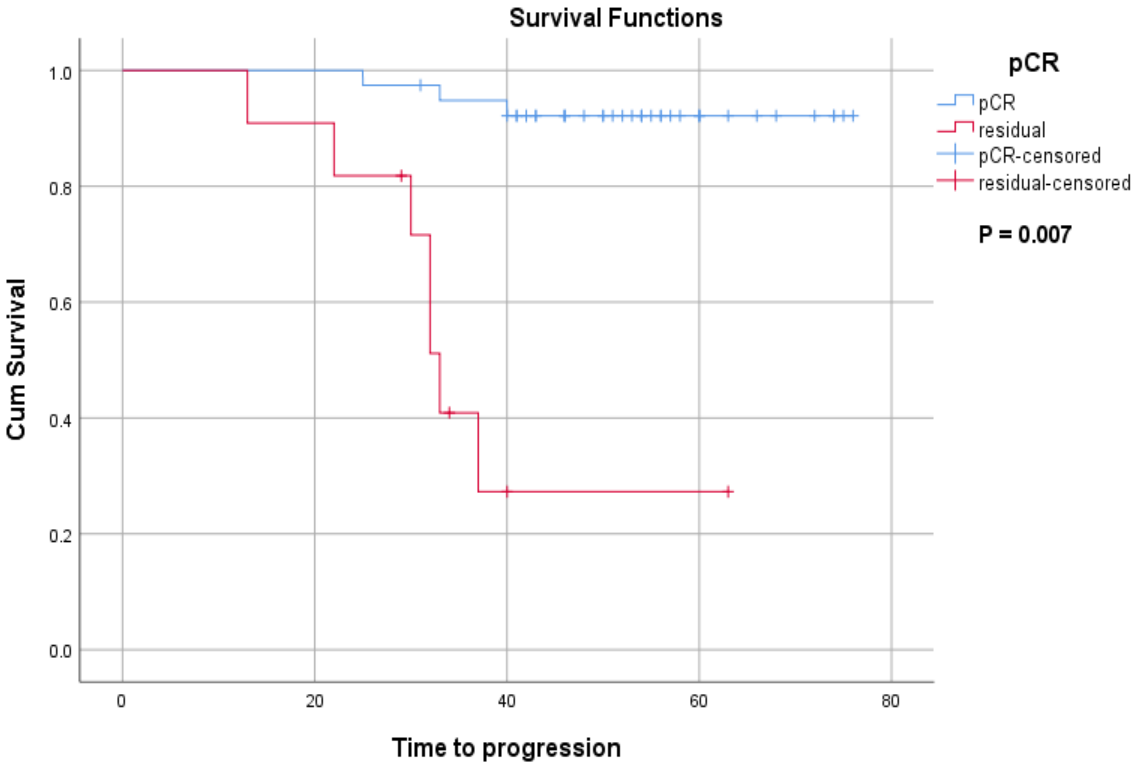

Figure 2 (b): The impact of pCR on PFS of TNBC.

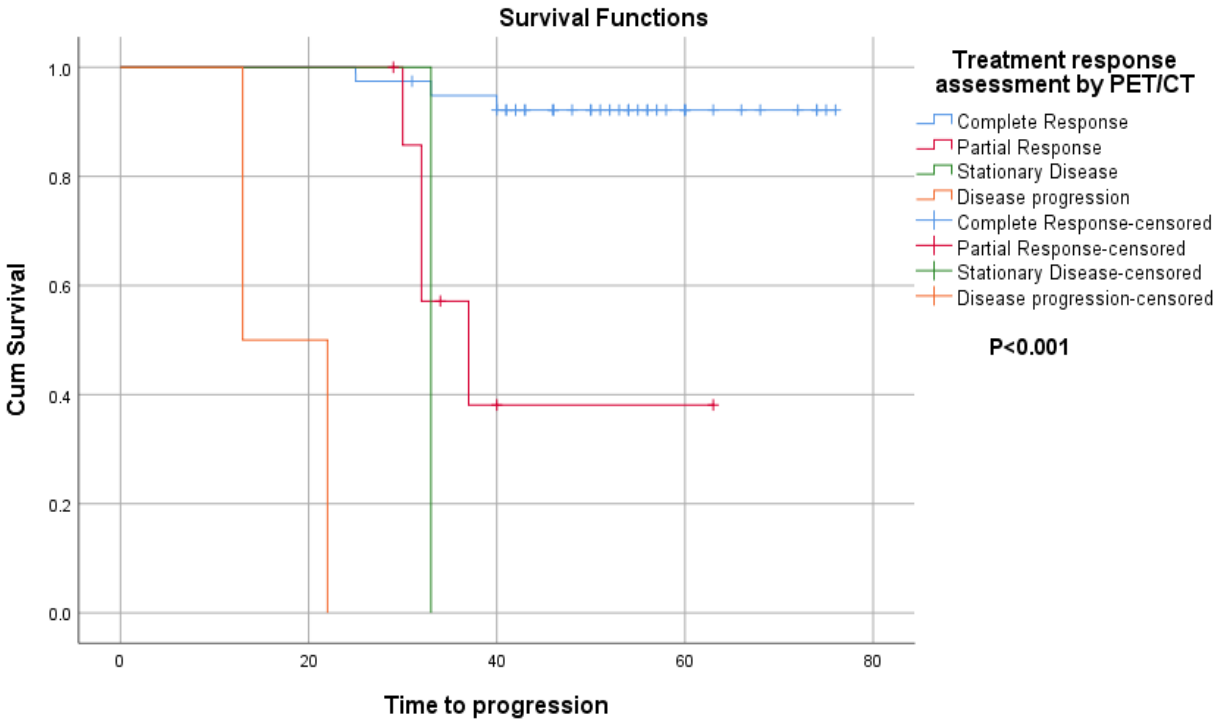

Figure 2 (c): PFS and PET/CT response assessment.

4. Conclusions

Overall survival issignificantly affected by tumor size (T) and high CK5/6, pCR and response on PET/CT. PET/CT results correlates with pCR, OAS, and PFS. Biomarkers and PET/CT may be used in the prognosis of tumor response in patients with TNBC who are undergoing NAC.

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