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**Background:** CA15-3, CA27.29, and CEA are currently being used as biomarkers in breast cancer (BC). Their performance is not satisfactory due to the low correlation between the biomarker levels and the treated patient's status. We have reported that blood Thioredoxin1 (Trx1) could be a specific biomarker to detect BC. Therefore, it would be interesting to study if the blood Trx1 level has the potential to be a monitoring biomarker for BC patients.

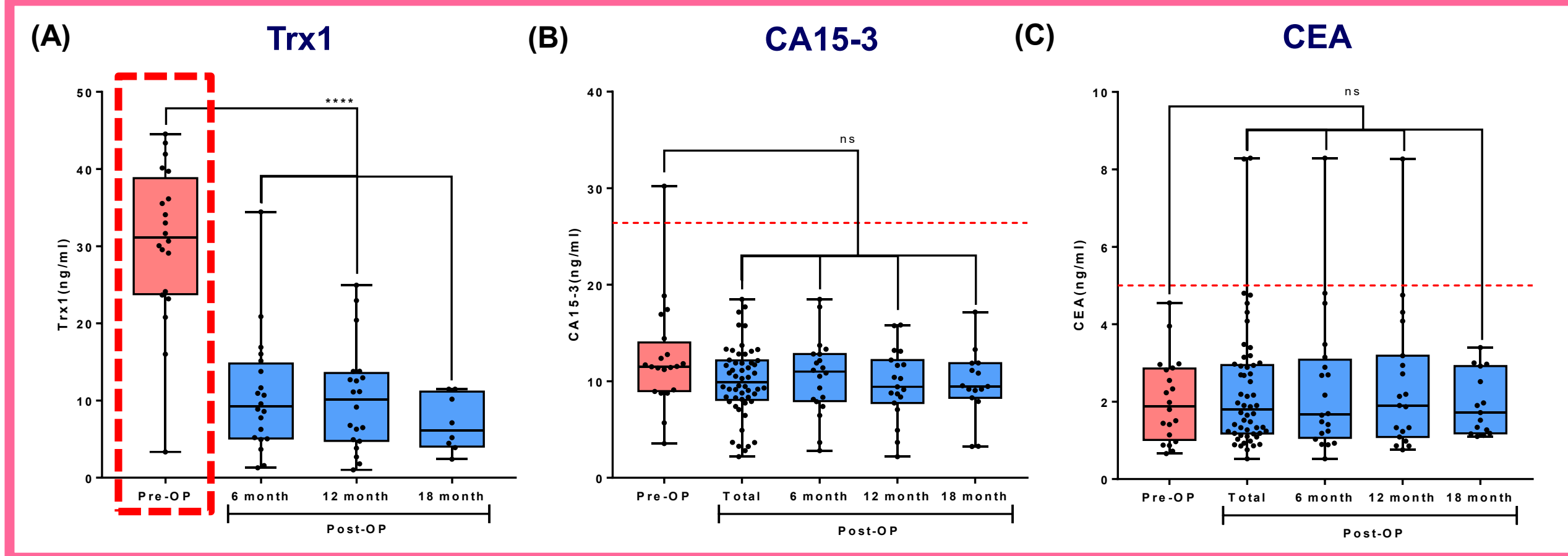
**Methods:** This study was approved by the IRB of the Chungnam National University Hospital. The blood from 20 patients was collected before operation and at the designated time points after the surgery such as 6, 12, and 18 months. We performed an immunoassay to quantify serum Trx1 (DxMe<sup>®</sup> BC kit). Analysis was retrospectively compared to those from clinical information and treatment.

**Results:** The mean level of Trx1 from BC patients before surgery was  $30.53 \pm 10.09$  ng/ml ( $\pm$ SD) that was higher than the preset cut-off value (11.4 ng/ml). The blood Trx1 levels from the patients at 6, 12, and 18 months after surgery were  $10.66 \pm 7.67$  ng/ml,  $10.2 \pm 6.81$  ng/ml, and  $7.02 \pm 3.60$  ng/ml, respectively, which were in the range of reference interval. Dramatic decreases in Trx1 levels were observed commonly from pre- to post-surgery. The decreased Trx1 levels seemed to be sustained at the low levels during the period of the study. In comparison, the serum levels of CEA and CA15-3 did not show any noticeable changes. Furthermore, the mean level of Trx1 after surgery was not affected by pathological and molecular features of BC or types of therapies. To evaluate the diagnostic ability of Trx1 for the BC patients underwent operation, we carried out the ROC analysis between pre- and post-operation cases. Comparing pre- and post-operation, the overall clinical sensitivity was 90.00% and clinical specificity was 91.67%.

**Conclusion:**

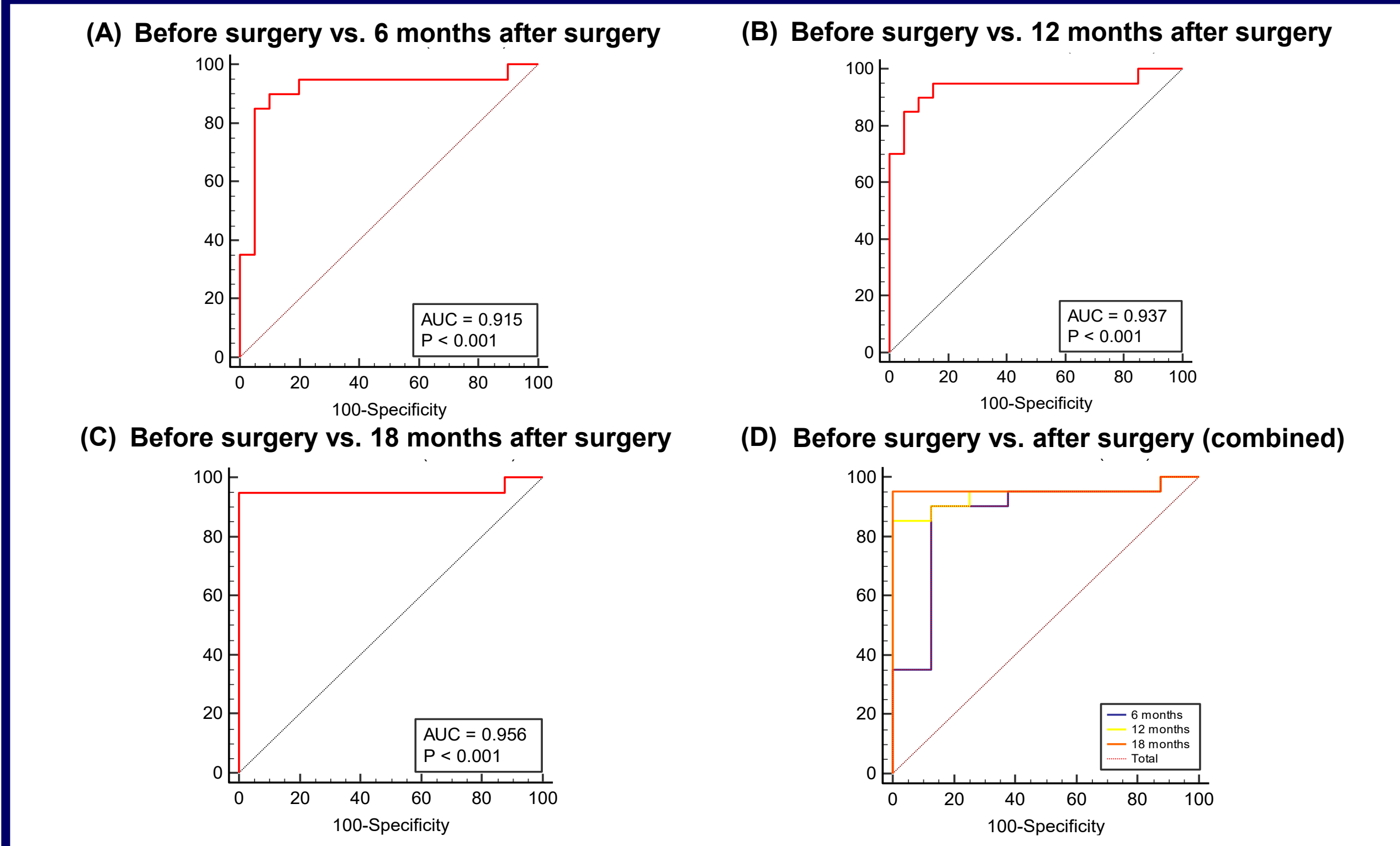
- The blood Trx1 level was decreased dramatically after surgery, and it was likely to depend on the presence or absence of a tumor mass.
- Consequently, the blood Trx1 level has potential as a monitoring biomarker to assist the management of BC patients after treatment.

## RESULTS



**Fig. 1. The blood level of Trx1, CA15-3, and CEA from BC patients during follow-up periods.**

The levels of each biomarker were analyzed and compared during follow-up period (pre-operation, 6, 12, 18 months). The levels of Trx1 of BC patients before surgery were higher than those of the treated patients at 6, 12, 18 months after surgery. In comparison, the levels of CEA and CA15-3 did not show any noticeable changes. (A) The box and whisker plot of Trx1 level during follow-up periods. (B) The blood level of CA15-3 (C) The blood level of CEA. Pre-OP, pre-operation; Post-OP, post-operation



**Fig. 2. ROC analysis of blood Trx1 levels in BC patients before operation compared with those from the same patients during follow-up periods after surgery.**

(A) ROC analysis to see how blood Trx1 level discriminates BC patients from those who were at 6 months after surgery. The clinical sensitivity and specificity were both 90.00% with of  $0.915 \pm 0.054$  AUC. (B) ROC analysis after 12 months. The clinical sensitivity and specificity were both 90.00% with  $0.937 \pm 0.045$  AUC. (C) ROC analysis after 18 months. The clinical sensitivity and specificity were 95.00%, 100.00%, respectively with  $0.956 \pm 0.044$  AUC. (D) Combined ROC analysis with all patients after surgery. The overall clinical sensitivity and specificity were 90.00%, 91.67%, respectively with  $0.931 \pm 0.045$  AUC.

**Table 1. Participants in this study**

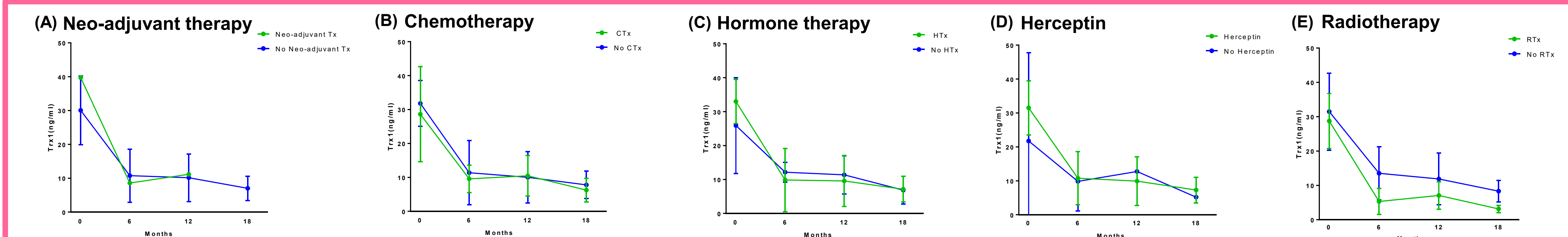
Patients' status			
Breast cancer status	No.	Trx1 (95% CI)	Min/Max
Pre-operation	20	30.53(25.81 to 35.25)	3.35/44.53
Post-operation (Total)	48	9.86(7.89 to 11.84)	1.02/34.43
Post-operation 6 months	20	10.66(7.07 to 14.25)	1.29/34.43
Post-operation 12 months	20	10.20(7.01 to 13.39)	1.02/24.94
Post-operation 18 months	8	7.02(4.01 to 10.03)	2.4/11.48

Patients' clinical information			
Variable	No.	Trx1 (95% CI)	%
Age	30s	24.81(13.83 to 35.80)	15%
	40s	34.20(21.65 to 46.74)	20%
	50s	34.19(30.35 to 38.02)	35%
	60s	22.92(6.58 to 39.26)	15%
	70s	23.94(NA)	10%
	80s	43.38(NA)	5%
Histologic subtypes	DCIS	32.65(27.26 to 38.04)	35%
	IDC	29.38(22.23 to 36.54)	65%
Histologic grade	1	29.62(25.12 to 34.11)	25%
	2	23.16(23.16 to 44.91)	25%
	3	29.23(20.02 to 38.44)	50%
TNM stage	0	32.65(27.26 to 38.04)	35%
	1A	26.91(17.48 to 36.34)	45%
	2A	35.53(NA)	5%
	2B	43.38(NA)	5%
Molecular subtypes	3C	30.46(NA)	10%
	Luminal A	29.69(26.28 to 33.10)	30%
	Luminal B	34.39(27.67 to 41.10)	40%
HER2-enriched	TNBC	29.97(8.35 to 51.59)	20%
	2	18.72(NA)	10%
Ki67	<15%	28.86(25.87 to 31.85)	40%
	≥15%	31.64(23.50 to 39.79)	60%
p53	1	27.17(13.64 to 40.69)	30%
	2	32.9(26.09 to 39.70)	30%
	3	30.01(20.04 to 39.98)	35%
	NA	40.13(NA)	5%

**Therapy information**

Therapy	No.	Trx1 (95% CI)	%
Neo-adjuvant therapy	1	39.71(NA)	5%
No neo-adjuvant therapy	19	30.04(25.16 to 34.92)	95%
Chemotherapy	8	28.62(16.87 to 40.37)	40%
No chemotherapy	12	31.80(27.50 to 36.10)	60%
Hormone therapy	13	33.01(29.08 to 36.93)	65%
No hormone therapy	7	25.92(12.85 to 38.99)	35%
Radiotherapy	7	28.76(21.35 to 36.18)	35%
No radiotherapy	13	31.48(24.69 to 38.27)	65%
Herceptin	2	21.74(NA)	10%
No Herceptin	18	31.50(27.53 to 35.48)	90%



**Fig. 3. The effect of type of BC therapy on the blood level of Trx1 during follow-up periods**

Trx1 levels from BC patients were analyzed by the type of BC therapy. (A) Effect of neo-adjuvant therapy of BC. (B) Effect of chemotherapy (C) Effect of hormone therapy (D) Effect of targeted therapy (E) Effect of radiotherapy