Original Article

Histomorphological Changes in Breast Cancer following Neoadjuvant Therapy and its Prognostic Implication for Patient Management — Study of 36 Cases in a Tertiary Care Hospital

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Background : Neoadjuvant Therapy is the systemic administration of therapeutic agent before definitive surgery. Neoadjuvant Therapy (NAT), specially Neoadjuvant Chemotherapy (NACT) is a standard procedure in locally advanced breast cancers to reduce the tumor size & down stage the disease leading to increase in the chances of successful resection. Following NAT there are various histomorphological changes in the breast cancer, both in the tumor cells & stroma. Our aim of the study was to evaluate the various histomorphological changes in breast cancer following neoadjuvant chemotherapy and its prognostic implications.

Materials and Methods : The study was conducted over a period of 4 years which included 36 cases of breast cancer receiving NACT. Various histomorphological parameters such as pathological response, presence of residual tumor, its grading, Tumor Infiltrating Lymphocytes (TILs), stromal changes such as fibrosis, collagenization, microcalcification, hemosiderin laden macrophages were studied.

Result : In our study patients were between 30-70 years of age. The most common histomorphological changes were nuclear enlargement and pleomorphic nuclei(28 cases, 77.7%). Pathological Complete Response (PCR) & Tumor Infiltrating Lymphocytes (TIL) seen in 8 & 16 cases (77.7%, 22.22%) respectively, in which both Overall Survival (OS) and Disease Free Survival (DFS) were seen at greater incidence. Statistical analysis was done by software IBM SPSS version 20.0.

Discussion & Conclusion : Pathological evaluation of post NACT surgical samples in breast cancer is extremely important for assessing treatment response. Prognostic value in post NACT breast cancer was directly related to tumor staging after NACT, TIL and pathological response. To conclude histopathological examination of the tumor bed to assess the residual tumor is the gold standard for assessing the response to NACT in breast cancer.

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Key words **: Neoadjuvant Therapy, Breast Cancer, Pathologic Response, Residual Tumor.**

According to GLOBOCAN 2020 female breast cancer is the most common cancer (11.7%) surpassing the lung cancer[1]. Neo Adjuvant Therapy (NAT) is an important modality for treatment of breast cancer. NAT is defined as systemic administration of therapy prior to surgical removal of a breast tumor[2]. Initially NAT was used only for locally advanced inoperable breast carcinoma. But nowadays NAT has also been used for the treatment of early stage breast cancers as well with different applications as follows :

(i) In advanced inoperable carcinomas to decrease in tumor size and downstage the disease to make It operable for better surgical outcome[3].

(ii) In early stage breast carcinoma to shrink the tumor and thus allowing breast conserving surgery [4].

(iii) In clinically node negative breast cancer patients with unfavorable tumor profiles in whom adjuvant systemic therapy is predicted, neoadjuvant therapy prior to surgery reduces the extent of axillary surgery[5].

(iv) Basing on the pathological response to NAT and the residual tumor burden it provides prognostic in formations such as decrease risk of distant metastasis, rate of DFS & OS[6]. The gold standard for assessing the response to NAT is pathological evaluation of surgical samples following NAT[7].

On histopathological examinations of post NAT samples Pathologic Complete Response (PCR) and Residual Cancer Burden (RCB) are the two most

Editor's Comment :

- Thorough knowledge of the histopathological changes both in the tumor cells and the stroma of post NACT Breast cancer specimensis of ardent need for the effective and planned regimen of therapy to enhance both the overall and disease free survival in these patients.

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important parameters which are independently associated with improved survival outcomes[8,9].

NAT includes Neoadjuvant Chemotherapy (NACT), radiotherapy and targeted hormonal therapy. In our study we have analyzed the response predominantly to NACT in breast cancer patients.

Objective

The objective was to study the various histomorphological changes in breast cancer (in the tumor cells and stroma) in response to NACT& correlation of the prognostic markers such as OS and DFS with the histomorphological parameters.

Materials and Methods

(A) Study design, duration & place of study – It was a retrospective study conducted over a period of 4 years from August, 2018 to August, 2022 in a tertiary care hospital of West Bengal.

(B) Inclusion Criteria — Breast cancer patients who had received NAT & having pre-NAT biopsy report along with post-NAT follow updetails were included in the study.

(C) Exclusion Criteria — Breast cancer patients in whom pre NAT biopsy report & follow-up details not available were excluded from the study.

(D) Detailed clinical history regarding clinical presentation, radiological findings, pre NAT biopsy diagnosis, NAT therapy cycles were taken from clinical records.

(E) Specimen handling —

i) Gross examination : Post NAT Modified Radical Mastectomy (MRM) and Breast Conservative Surgery (BCS) specimens were examined in fresh state after receiving to identify and measure the tumor bed ie, the tissue encompassing the original tumor site[10]. In cases of complete response to NAT it is difficult to identify any grossly visible lesion. In cases of partial response or no response to NAT, size and number of any residual disease foci are examined. The distance of the tumor bed or residual tumor, from the surgical margins were noted in case of BCS specimens.

ii) Fixation, Grossing & Sectioning : Fixation done in 10% formalin and sections were taken. Following the Food Drug Administration (FDA) recommendations (a minimum of 1 block per centimeter of pre-NAT tumor size or at least 10 blocks in total whichever is greater) sectioning is done in MRM/BCS specimens[11]. In our study axillary lymph node sampling were done following the standard operating procedures[12].

iii) Tissue Processing : All the sectioned tissue bits were processed, stained by Hematoxylin & Eosin (H&E) Stain and examined underthe microscope.

iv) Histological Examination : Things looked for -

(a) Presence of any residual invasive cancer component,histologic subtype & grade. Presence of in-situ component, lymphovascular invasion, necrosis, calcification, number of positive lymph nodes, and status of surgical margins (in BCS) were also noted.

(b) Stromal changes such as hyalinization, foamy macrophages, lymphocytes,multinucleated giant cells, hemosiderin-laden macrophages, necrosis and micro-calcification were studied.

v) Pathological staging after NAT were done based on the TNM staging system (8th. Edition of AJCC)

(F) Statistical Analysis — Soft ware SPSS version 20.0 was used for data analysis and all thedata were represented as number (n) and percentage (%) and compared by X^2 test as applicable.

(G) P Value — <0.05 was considered statistically significant.

(H) Ethical Clearance — Ethical clearance was taken from Institutional Ethical committee (Figs 1-4).

Result

In our study a total of 36 cases of breast cancer receiving NACT were studied. Various histo-

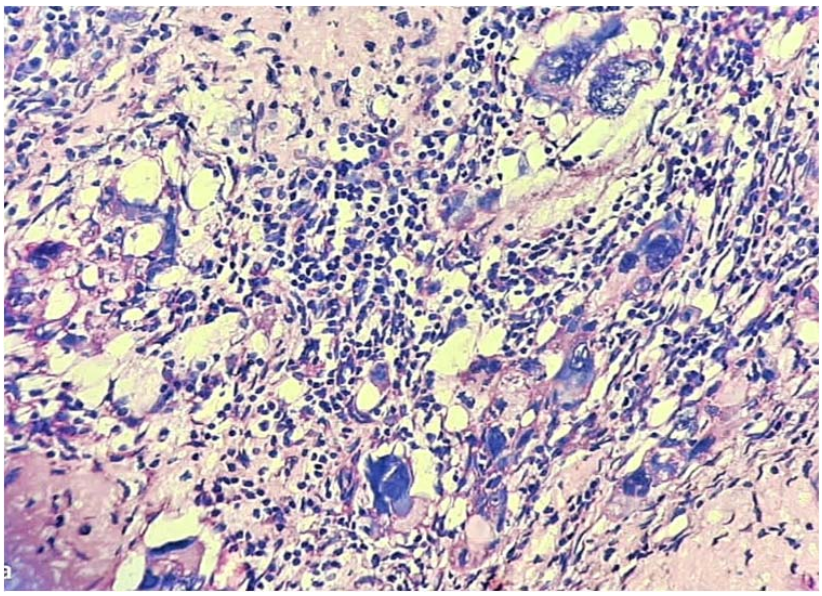

Fig 1 — Photomicrograph showing tumor cells with pleomorphic, bizzare nuclei & TILs in breast cancer following NACT (H&E,100X)

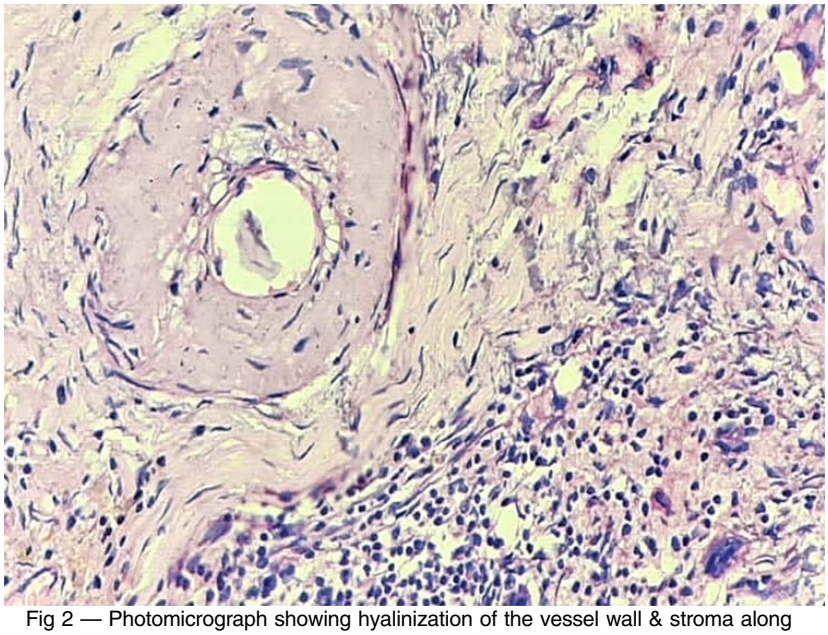

Fig 2 — Photomicrograph showing hyalinization of the vessel wall & stroma along with TILs in breast cancer following NACT (H&E,100X)

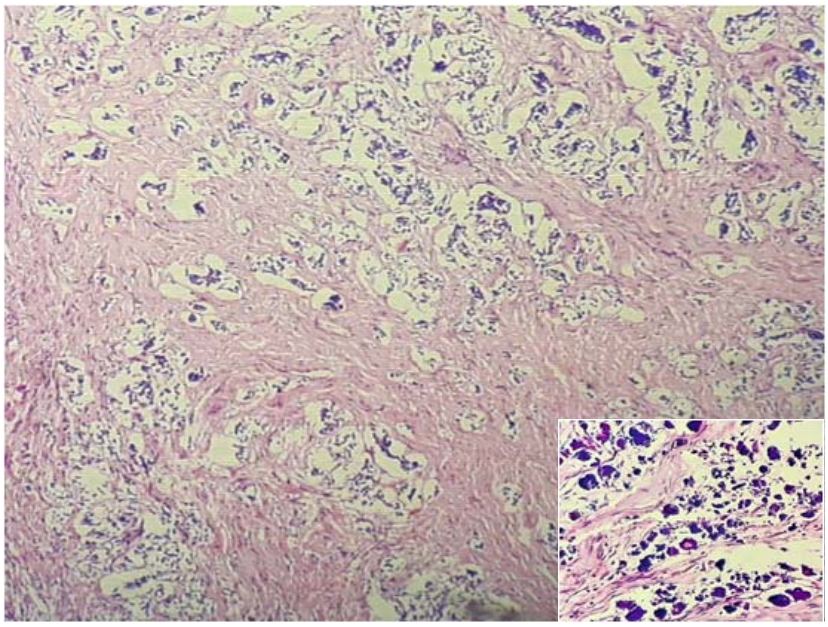

Fig 3 — Photomicrograph showing downgraded residual tumor in breast cancer& calcification (inset) following NACT (H&E, 100X)

morphological changes seen were as follows. Nuclear enlargement- 28 cases (77.7%), hyperchromasia- 26 (72.22%), pleomorphic nuclei- 28 (77.7%), karyorrhexis / karyolysis- 13 (36.11%). Stromal response - fibrosis in 12 cases (33.33%), collagenization-3 cases(8.33%), calcification in 5 cases(13.88%), lymphocytic infiltrate in 11 cases (30.55%), foamy histiocytes in 2 cases (5.55%), giant cells in 3 cases (8.33%) and Hemosiderin laden macrophages in 2 cases (5.55%)(Table 1).

In our study PCR was seen in 8 cases (22.22%) & rest 28 cases showed PPR(pathological partial response)-(6 cases) & PNR (pathological no response)- (22 cases) respectively.

Most common type of residual cancer seen was Invasive Carcinoma of No Special Type (27,75%)(Tables 2&3). In our cases pre-NAT TIL seen in 16 cases. OS & DFS free survival seen in 11 cases. Post NAT stromal lymphocytes seen in 11 cases out which7 cases had PCR (Table 4).

Discussion

Neo Adjuvant Therapy (NAT) is defined as administration of therapeutic agents prior to definitive surgery in Breast cancer patients. NAT may be Neoadjuvant Chemotherapy (NACT) or radiotherapy or targeted hormone therapy. In contrast to other studies in which some breast cancer patients received endocrine therapy and targeted therapy korde LA, *et al*[7] in our study, We have analyzed the breast cancer patients who received NACT. A total of 36 cases of post NACT breast cancer patients were studied. There were various morphologic alterations both in breast cancer cells and in the stroma following NACT. Different studies have shown the benefits of NACT in breast cancer[13].

In our study the age of the patients range from 30-70 years, similar to study by Cheryl Sarch Phillipose, *et al*[14].

In our study clinical findings such as tumor size, quadrant involved, axillary lymph node status along with pre-NAT radiological findings (mammography & USG), pre-NAT biopsy diagnosis, hormonal status (ER, PR, HER-2 neu)were all collected from clinical records.

Pathologic response following NAT were defined as follows :

According to Chevallier System[15]

PCR- Disappearance of all the tumor or DCIS in breast with no Invasive Carcinoma and negative lymph node.

PPR- presence of invasive carcinoma alongwith stromal alterations.

Table 1 — *Nuclear changes and stromal response in carcinoma breast following NACT (n=36)*

A. Nuclear changes	Number	Percentage(%)
i) Nuclear enlargement	28	77.77%
ii) Hyperchromasia	26	72.22%
iii) Pleomorphic nuclei	28	77.77%
iv) Increased nucleocytoplasmic ratio	23	63.88%
v) Karyorrhexis/lysis	13	36.11%
B. Stromal response	**Number**	**Percentage(%)**
i) Fibrosis/desmoplasia	12	33.33%
ii) Elastosis/collagenization	03	8.33 %
iii) Calcification	05	13.88%
iv) Foamy histiocytes	02	5.55 %
v) Giant cells	03	8.33 %
vi) Hemosiderin laden macrophages	02	5.55%
vii) Lymphoplasmacytic infiltrate	11	30.55%

Table 2 — *Pathologic response in carcinoma breast following NACT(n=36)*

Response	Number	Percentage(%)
i) PCR	08	22.22%
ii) PPR/PNR	28	77.77%
a) Histologic type		
- Invasive carcinoma No special type	27	75%
- Invasive Lobular carcinoma	1	2.7%
b) Post histologic grade(Modified Bloom – Richardson) Grade		
• 0	08	22.2%
• 1	06	16.66%
• 2	22	61.11%
• 3	22	61.11%
iii) Presence of DCIS		
- Yes	9	25%
- No	27	75%
iv) Lymphnode tumor deposit		
Yes	16	44.44%
No	20	55.55%

Table 3 — *Effect of NACT on carcinoma breast comparison with other studies*

Effect	Sethi, *et al* n=40(%)	Sheereen, *et al* n=39(%)	In our study n=36(%)
PCR	4/40 (10%)	7/39 (17.6%)	8/36 (22.22%)
PPR	12/40 (30%)	6/39 (15.4 %)	6/36 (16.66%)
PNR	24/40 (60%)	26/39 (66.7%)	22/36 (61.0%)

Table 4 — *Correlation of disease free survival with RDTILs & pathologic response (n=36)*

Parameters		DFS	P Value
1) Presence of RD TIL	Yes = 11	9	<0.001
	No = 25	12	<0.001
2) PCR	Yes = 8	8	< 0.001
	No =-28	28	< 0.001

PNR- little modification in the originaltumor appearance.

According to AJCC[16] treatment Effect in the Breast:

- No definite response to NAT.
- Probable or definite response to NAT.
- No residual invasive carcinoma is present in the breast.

Treatment Effect in the lymphnode :

- No definite response.
- Probable or definite response.
- No lymph node metastasis seen only fibrous scarring.

According to this PCR was defined as ypTo/Tis ypNo.

Similar to other studies[17] in our study there were various nuclear and cytoplasmic alteration seen which include nuclear enlargement,nuclear Karyorrhexis, pyknosis, cytoplasmicvacuolization etc. Among these nuclear enlargement and pleomorphism were the most common findings.

In PCR microscections show only stromal fibrosis, giant cells, lymphocytes and microcalcifications.

DCIS were seen 9 cases (25%) (Table 2) similar to study by DhanyaVasudevan, *et al*[17].

Out of 36 in 8 cases (22.22%) PCR was seen, similar to the study by Sheereen, *et al*[18]and Sethi, *et al*[19] (Table 3).

In our study in 20 cases (55%) axillary lymph nodes, show tumor deposit (Table 2), majority of them (18 cases) show tumor grading similar to Pre-NAT tumor grading.

Prognostic significance of histomorphological parameters.

(a) Tumor staging after NAT :

More than 15 different systems have been proposed in the last 30 years for categorizing NAT response[20]. Comprehensive systems include clinical (pre-NAT) and pathological (Post-NAT) stage, tumor grade, hormonal status (ER, PR, HER-2 status) (i.eneobioscoring staging system)[21] or tumor bed area, cellularity of residual invasive cancer, ki-67 labeling index, number of positive nodes and size of the largest metastasis (ie, residual proliferative cancer burden)[22]. Whatever may be the tumor staging system, parameters that are associated with improved DFS and OS) are included in it.

(b) TILs-Evaluation of TILS in the post-NAT breast tissues having residual disease is gradually gaining increasing importance[23]. In our study it was seen higher RD TIL levels were significantly associated with improved DFS & OS. This suggest RD-TIL indicates heterogenous immune responses to NACT and an independent prognostic implications to markers of tumor response[24].

(c) Pathologic response– As described earlier PCR is a well established end point of NACT. PCR is an useful prognostic marker as it is independently associated improved survival outcomes compared with patients without PCR similar to study by Cortazar P, *et al*[8], in our study patients with PCR had shown better DFS & OS.

Limitation of the Study :

The limitation of the study includes

(1) Small sample size

(2) Data on re evaluation of biomarker status, ER, PR, HER -2 after NAT was not available .

Conclusion

Since NAT is now an established effective treatment for breast cancer, the number of post NAT specimens has recently increased. As NAT cause diverse range of histological alterations a thorough knowledge of the cytologic & stromal changes rendered by therapy is necessary & extremely important for correct diagnosis, grading of tumor leading to an effective & planned regimen of therapy. This ultimately leads to better clinical outcome & effective patient management. To conclude pathological evaluation of residual disease is the most essential component of post -NAT breast specimens.

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Conflicts of Interest : none.

References

1 Deo SVS, Sharma J, Kumar S — GLOBOCAN 2020 Report on Global Cancer Burden: Challenges and Opportunities for Surgical Oncologists. *Ann Surg Oncol* 2022; **29:** 6497-500. https://doi.org/10.1245/s10434-022-12151-6.

2 Masood S — Neoadjuvant chemotherapy in breast cancers. Womens Health (Lond). 2016 Sep;12(5):480-491. doi: 10.1177/1745505716677139. PMID: 27885165; PMCID: PMC5373271.

3 National Comprehensive Cancer Network. NCNN clinical practice guidelines in Oncology- breast cancer. 2020. Version 4.

4 Franceschini G, Di Leone A, Natale M, Sanchez MA, Masett R — Conservative surgery after neoadjuvant chemotherapy in patients with operable breast cancer. *Ann Ital Chir* 2018; **89:** 290.

5 Vugts G, Maaskant-Braat AJ, Nieuwenhuijzen GA, Roumen RM, Luiten EJ, Voogd AC — Patterns of Care in the Administration of Neo-adjuvant Chemotherapy for Breast Cancer. *A Population-Based Study. Breast J* 2016; **22(3):** 316-21. doi:10.1111/tbj.12568

6 Greenwell K, Hussain L, Lee D, Bramlage M, Bills G, Mehta A, *et al* — Complete pathologic response rate to neoadjuvant chemotherapy increases with increasing HER2/CEP17 ratio in HER2 overexpressing breast cancer: analysis of the National Cancer Database (NCDB). *Breast Cancer Res Treat* 2020; **181:** 249-54.

7 Korde LA, Somerfield MR, Carey LA, Crews JR, Denduluri N, Shelley Hwang E, *et al* — Neoadjuvant Chemotherapy, Endocrine Therapy, and Targeted Therapy for Breast Cancer: ASCO Guideline. *Journal of Clinical Oncology* 2021; **39(13):** 1485-505. doi: 10.1200/JCO.20.03399

8 Cortazar P, Zhang L, Untch M, Mehta K, Constantino JP, Wolmark N, *et al* — Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis [published correction appears in Lancet. 2019 Mar 9;393(10175):986]. *Lancet* 2014; **384(9938):** 164-72. doi:10.1016/S0140-6736(13)62422-8

9 Symmans WF, Peintinger F, Hatzis C, Rajan R, Kuerer H, Valero V, *et al* — Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J ClinOncol. 2007; **25(28):** 4414-22. doi:10.1200/JCO.2007.10.6823

10 Viale G — Characterization and clinical impact of residual disease after neoadjuvant chemotherapy. *Breast* 2013; **22:** S88-91.

11 Cortazar P, Geyer CE — Pathological complete response in neoadjuvant treatment of breast cancer. *Ann Surg Oncol* 2015; **22:** 1441-6.

12 Fitzgibbons P, Connolly J, Bose S. Pathologists CoA — Protocol for the examination of resection specimens from patients with invasive carcinoma of the breast. College of American Pathologists Version: Breast Invasive Resection, 2020. 4.4.0.

13 Sahoo S, Lester SC — Pathology of breast carcinomas after neoadjuvant chemotherapy. *Arch Pathol Lab Med* 2009; **133:** 633-42.

14 Philipose CH, Umashankar T, Gatty RC — A Histo-Morphological Study of Changes in Neoadjuvant Chemotherapy in Breast Malignancies. *JCDR* 2019; **13(3):** EC15-EC18.

15 Chevallier B, Roche H, Olivier JP, Chollet P, Hurteloup P — Inflammatory breast cancer. Pilot study of intensive induction chemotherapy (FEC-HD) results in a high histologic response rate. *Am J Clin Oncol* 1993; **16(3):** 223-8.

16 Cho SY, Park SY, Bae YK, Kim JY, Kim EK, Kim WG, *et al* — Standardized Pathology Report for Breast Cancer. J Breast Cancer 2021; **24(1):** 1-21. doi:10.4048/jbc.2021.24.e5

17 Vasudevan D, Jayalakshmy PS, Kumar S, Mathew S — Assessment of Pathological Response of Breast Carcinoma in Modified Radical Mastectomy Specimens after Neoadjuvant Chemotherapy. *Int J Breast Cancer* 2015; 2015: 536145. doi:10.1155/2015/536145

18 Sheereen S, Lobo FD, Kumar B, Kumar MS, Reddy SG, Patel W, *et al* — Histopathological Changes in Breast Cancers Following Neoadjuvant Chemotherapy: Implications for Assessment of Therapy-Induced Cytological and Stromal Changes for Better Clinical Outcome and Effective Patient care. *Asian J Oncol* 2018; **4:** 61-8.

19 Sethi D, Sen R, Parshad S, Khetarpal S, Garg M, Sen J — Histopathologic changes following neoadjuvant chemotherapy in locally advanced breast cancer. *Indian J Cancer* 2013; **50(1):** 58-64

20 Mrkonjic M, Berman HK, Done SJ, Youngson B, Mulligan AM — Breast specimen handling and reporting in the post-neoadjuvant setting: challenges and advances. J ClinPathol 2019; **72:** 120-32.

21 Mittendorf EA, Vila J, Tucker SL, Chavez-MacGregor M, Smith BD,Symmans WF, *et al* — The neo-bioscore update for staging breast cancer treated with neoadjuvant chemotherapy: incorporation of prognostic biologic factors into staging after treatment. *JAMA Oncol* 2016; **2:** 929-36.

22 Sheri A, Smith IE, Johnston SR, A'Hern R, Nerurkar A, Jones RL, *et al* — Residual proliferative cancer burden to predict long-term outcome following neoadjuvant chemotherapy. *Ann Oncol* 2015; **26:** 75-80.

23 Dieci MV, Radosevic-Robin N, Fineberg S, van den Eynden G, Ternes N, Penault-Llorca F, *et al* — Update on tumor-infiltrating lymphocytes (TILs) inbreast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: a report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer. *Semin Cancer Biol* 2018; **52:** 16-25.

24 Criscitiello C, Bayar MA, Curigliano G, Symmans FW, Desmedt C, Bonnefoi H, *et al* — A gene signature to predicthigh tumor-infiltrating lymphocytes after neoadjuvantchemotherapyand outcome in patients with triple-negative breast cancer. *Ann Oncol* 2018; **29(1):** 162-9.