Comparison of Tumor - Infiltrating Lymphocytes Between Primary and Metastatic Tumors in Her2+ and HER2-Breast Cancer Patients

CLAUDIA MEHEDINTU1, ELVIRA BRATILA1, COSTIN BERCEANU2, MONICA MIHAELA CIRSTOU1, RAMONA ILEANA BARAC1, CRISTINA VERONICA ANDREESCU1, DUMITRU CRISTINEL BADIU1, LAURENTIA GALES1, ANCA ZGURA1, ADRIAN GHEORGHE BUMBU1

1 Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd., 050474, Bucharest, Romania
2 University of Medicine and Pharmacy, 2 Petru Rareș Str., 200349, Craiova, Romania
3 University of Oradea, Faculty of Medicine and Pharmacy, 10, 1 Decembrie Sq., 410068, Oradea, Romania

The impact of tumor infiltrating lymphocytes (TILs) on survival was confirmed in various cancer types. Our study aims to investigate the prognostic role of TILs on survival in patients with primary and metastatic tumors in breast cancer patients. We retrospectively identified 29 patients with human epidermal growth factor receptor-2 (HER2+) and HER2 - early breast cancer diagnosed between 2012 and 2018 at Institute of Oncology Prof. Dr. Al. Trestioreanu Bucharest and who subsequently experienced regional or distant recurrence confirmed by tumor biopsy/resection.

Keywords: metastatic breast tumor, primary breast tumor, tumor-infiltrating lymphocytes, HER2, immune system

Breast cancer (BC) is the second leading cause of cancer death in women [1,2]. It has been estimated that half of the new worldwide BC cases and 60% of the BC deaths occur in developing countries [3]. It occurs more frequently in elderly women and in most cases, risk factors are linked to estrogen hormone that stimulates breast tumor growth [1,4-8]. Tumor development is a heterogeneous process, making it difficult to evaluate the relationship between the tumor and the tumor microenvironment [9]. In breast cancer, the adaptive immune response can easily be seen in the infiltration of breast lesions from the time of benign breast atypia and with the increase in density due to invasive malignancy [10]. The presence of tumor-infiltrating lymphocytes (TILs) is associated with favorable with good prognostic in breast cancer [11,12]. TILs play an important role in mediating the response to chemotherapy and in improving all clinical outcomes in most subtypes of breast cancer [10].

Previous studies have reported that immune activation at the baseline, as assessed by pathology or gene expression arrays, is associated with a higher likelihood of pathological complete response after neoadjuvant chemotherapy (NAC) [13,14], particularly in human epidermal growth factor receptor-2 (HER2)-positive and HER 2 negative breast cancers [15,16]. Furthermore, trastuzumab has been predicted to have beneficial effects. The presence of TILs in residual disease after neoadjuvant chemotherapy is associated with better prognosis breast cancers patients with HER2 negative [17-20]. This suggests that chemotherapy could convert low-TILs tumors into high-TILs tumors [21]. In the case of HER2 positive, even incremental increases in TILs inside and around the tumor were predictive in both the chemotherapy response and the improvement in patient survival rates [21-23]. This finding supports the concept that chemotherapy could partly exert its antitumor effect through the immune system [24]. Very little is known about the change in TILs during metastatic progression and the prognostic impact of TILs in metastatic sites [25,26].

Our study aims to investigate the prognostic role of TILs on survival in patients with primary and metastatic tumors in breast cancer patients.

Experimental part

We retrospectively identified 29 patients with HER2- and HER2+ breast cancer diagnosed between 2012 and 2018 at Institute of Oncology Prof. Dr. Al. Trestioreanu Bucharest and who subsequently experienced a regional or distant recurrence confirmed by tumor biopsy/resection.

All the tumor specimens were prepared for H&E staining and immunohistochemistry (IHC) and were reviewed by a pathologist. Immunohistochemistry was carried out using the following primary antibodies: anti estrogen receptor (ER), PR receptor and proliferation marker (Ki67). The specimens were considered positive for hormone receptor if ≥1% of the cancer cells expressed ER. For the patients determined as HER2+ by IHC, FISH was used to confirm HER2+ disease.

The breast cancer subtypes were classified using IHC as previously described: HER2 positive or HER2 overexpressing (HER2+). Hematoxylin–eosin stained slides for the paired match cases were evaluated for stromal TILs by a pathologist. The specimens were classified into three groups: low TILs (<10%), moderate TILs (10-60%), and lymphocyte predominant breast cancer (LPBC) (≥60%).

Statistical analyses

Associations of the percentage of between the primary and metastatic tumors were evaluated using Fisher’s exact test for categorical variables and using the two-sided t-tests for continuous variables. The correlation between the percentages of TILs was calculated using Spearman’s and Kendall’s rank correlation coefficient test. In all the analyses, the differences were considered significant at P < 0.05.

The research meets the conditions of the ethical guidelines and legal requirements and was approved by each Ethical Committee of the Universities of Medicine

* email: mediana@gmail

All the authors have equal contribution at this paper.
and Pharmacy (see authors’ affiliations). Informed consent was obtained from every patient included in the study.

Results and discussions

Medical files of 80 women diagnosed with breast cancer between 2010-2018 were retrospectively analyzed but only 29 breast cancer patients presented the inclusion criteria in the study. Median age at diagnosis was 59.78 years, stage distribution was 10% IA, 14% in stage IIB, 14% stage II A, 10% stage IIIB, 14% stage IIIC, and 38% stage IV.

| Characteristics     | Total patients (n=29) | HER2+ (n=11) | HER2- (n=18) |
|---------------------|-----------------------|--------------|--------------|
| Age, years          | 59.78                 | 71.5         | 53.53        |
| Mean (range)        |                       |              |              |
| T                   | 2                     | 0            | 2            |
| 1                   | 8                     | 3            | 5            |
| 2                   | 12                    | 4            | 8            |
| 3                   | 4                     | 0            | 4            |
| 4                   | 3                     | 3            | 0            |
| N                   | 14                    | 7            | 7            |
| 2                   | 2                     | 1            | 1            |
| 3                   | 5                     | 2            | 3            |
| Stage               | 3                     | 1            | 2            |
| 1                   | 9                     | 3            | 6            |
| 3                   | 7                     | 3            | 3            |
| 4                   | 10                    | 3            | 7            |
| Histological grade  | 0                     | 0            | 0            |
| 2                   | 17                    | 8            | 9            |
| 3                   | 12                    | 3            | 9            |
| ER                  | +                     | 24           | 11           | 13           |
|                    | -                     | 5            | 0            | 5            |
| Ki67                | 25.59%                | 13%          | 31.95%       |
| Chemotherapy        | Neoadjuvant           | 19           | 8            | 11           |
|                     | Adjuvant              | 5            | 1            | 4            |
| Trastuzumab         | YES                   | 3            |              |              |
|                     | NO                    | 26           |              |              |
| Hormonal therapy    | YES                   | 22           | 10           | 12           |
|                     | NO                    |              |              |              |

Table 1
AGE DIFFERENCE BETWEEN HER GROUPS

| Levene's Test for Equality of Variances | t-test for Equality of Means | 95% Confidence Interval of the Difference |
|----------------------------------------|-----------------------------|---------------------------------------|
| F                                      | Sig.                        | t                                      | df | Sig (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Age                                    | Equal variances assumed     | 8.020                                  | .007 | 3.604          | 27       | .001                   | 17.974 | 4.987  | 7.741 | 28.206 |
|                                       | Equal variances not assumed | 4.438                                  | .2683 | .000           | 26.803  | .000                   | 17.974 | 4.056  | 9.660 | 26.287 |

Table 2
CLINICOPATHOLOGICAL CHARACTERISTICS OF PRIMARY SURGICAL BREAST TUMOR SPECIMENS
Of all breast cancer patients, 38% represented HER2 positive patients and 62% HER2 negative patients, 5 patients are TNBC. Ki 67 percentage ranged between 1% and 80% (median was 25.59%). We didn’t find any statistical correlation between age and Ki67 level (fig.1). As reported in table 1 young patients had HER2+. The characteristics of the 29 breast cancer patients at the time of diagnosis of the primary breast cancer are presented in table 2. We evaluated the core needle biopsy and surgical specimens before received neoadjuvant chemotherapy for excluding the possibility of alterations in the immune microenvironments of the tumors caused by the therapy.

Most of the patients received neoadjuvant systemic treatment (85% of patients received chemotherapy, 27% of HER2+ patients received trastuzumab). The first biopsy sites of the metastatic tumors were the skin (n=2), brain (n=2), lung (n=5), bone (n=1), and liver (n=3). The median follow-up time after the first biopsy of recurrent tumors was 12 months (range, 2–36 months). One patient died of metastatic disease at the last follow-up.

Median TILs levels in the overall population were intermediate, with similar results in HER2- and HER2+ patients. Of the primary tumors, 7% were higher, 48% were intermediate TILs tumors, and 45% were low TILs tumors (table 3). Among the corresponding first metastatic tumors, 45% were intermediate TILs tumors and 55% were low TILs tumors. We found in our study that younger women showed lower levels of TILs than older patients. We have not found significantly different TILs levels between primary and metastasis in the 10 cases with available samples, we explored (table 4). In the group of patients studied, it found that tumors with intermediate TIL levels tended to have ER-negative/HER2-negative breast cancer.

The percentage of TILs in the primary tumors was not significantly different than in the metastatic tumors. This difference was similar in the human epidermal growth factor receptor 2 HER2+ and HER2- breast cancer groups. TILs were not significantly different in cases where the patient has been received prior neoadjuvant systemic treatment.

Impact of TILs on survival are presented in table 6.

### Table 3
DISTRIBUTION OF PATIENTS REGARDING TILs LEVELS FOR HER2- AND FOR HER2+

| TILs     | HER2+ | HER2- |
|----------|-------|-------|
| Low      | 40    | 42.1  |
| Intermediate | 50  | 47.4  |
| High     | 10    | 5.3   |

### Table 4
TUMOR-INFILTRATING LYMPHOCYTES (TILs) BETWEEN PRIMARY AND METASTATIC BREAST CANCER TUMORS FOR EACH SUBTYPE

| Subtype | First site of biopsy | Primary tumor TILs | Metastatic tumor TILs |
|---------|----------------------|--------------------|-----------------------|
| HER2+   | Lung                 | Low                | Low                   |
|         | Liver                | Low intermediate   | Low intermediate      |
|         | Brain                | Low                | Low                   |
|         | Lung                 | Low intermediate   | Intermediate          |
|         | Skin                 | Intermediate       | Intermediate          |
|         | Bone                 | Intermediate       | Low                   |

### Table 5
CORRELATIONS BETWEEN ANALYZED VARIABLES

Kendall's tau_b

| Variable | TILS1 Correlation Coefficient | TILS2 Correlation Coefficient |
|----------|-------------------------------|-------------------------------|
| varsta   | 1.000                         | 0.000                         |
| TILS1    | -.057                         | -.230                         |
| HER      | -.479                         | -.101                         |
| KI67     | -.101                         | 1.000                         |
| ER       | -.213                         | -.004                         |
| TILS2    | -.112                         | -.120                         |

Spearman's rho

| Variable | TILS1 Correlation Coefficient | TILS2 Correlation Coefficient |
|----------|-------------------------------|-------------------------------|
| varsta   | 1.000                         | 0.000                         |
| TILS1    | -.068                         | -.227                         |
| HER      | -.573                         | -.105                         |
| KI67     | -.105                         | 1.000                         |
| ER       | -.227                         | -.182                         |
| TILS2    | -.137                         | -.224                         |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Tumor-infiltrating lymphocytes are associated with a better neoadjuvant chemotherapy response and prognosis in HER2+ and HER2- breast cancers. Most of TILs levels on metastasis were low and did not differ between HER2- and HER2+ tumors. Younger patients showed significantly lower TILs. In TNBC patients, TILs were intermediate compared with HER2+ patients. The relationship between the immune system and HER2+ and HER2- breast cancer deserve further exploration in the metastatic settings.

### References

1. OGIYA, R., NIIKURA, N., KUMAKI, N., BIANCHINI, G., KITANO, S., IWAMOTO, T., et al., Cancer sci., 107, no. 2, 2016, p. 1730.
2. SAVAS, P., SALGADO R, DENEKERT, C., SOTIRIOU, C., DARCY, P.K., SMYTH, M.J., et al., Nat. Rev. Clin. Oncol., 13, no. 4, 2016, p. 228.
3. DIECI, M.V., CRISCTIELLO, C., GOUBAR, A., VIALE, G., CONTE, P., GUARNERI, V., et al., Ann. Oncol., 25, no. 3, 2014, p. 611.
4. TIT, D.M., BUNGAU, S., IOVAN, C., NISTOR CSEPPENTO, D.C., ENDRES, L., SAVA, C., SABAU, A.M., FURAU, G., FURAU, C., JCM, 7, no. 10, 2018, p. 297.
5. TIT, D.M., PALLAG, A., IOVAN, C., FURAU, G., FURAU, C., BUNGAU, S., Iran. J. Public Health, 46, no. 11, 2017, p. 1128.
6. DIECI, M.V., TSVETKOVA, V., ORVIETO, E., PIACENTINI, F., FICARRA, G., et al., Breast Cancer Res., 20, 2018, p. 62.
7. BUMBU, A., PASCA, B., TIT, D.M., BUNGAU, S., BUMBU, G., Farmacia, 64, no. 3, 2016, p. 419.
8. BUMBU, A., NACER, K., BRATU, O.; et al., Proceedings of the 14th National Congress of Urogynecology and the National Conference of the Romanian Association for the Study of Pain, 2017, p. 82.
9. SALGADO, R., DENEKERT, C., DEMARIA, S., SIRTAINE, N., KLAUSCHEN, F., PRUNERI, G., et al., Ann. Oncol., 26, no. 2, 2015, p. 259.
10. STANTON, S.E., DISIS, M.L., J. Immunother. Cancer., 4, 2016, p. 59.
11. LOI, S., SIRTAINE, N., PIETTE, F., SALGADO, R., VIALE, G., VAN EENO, F., et al. BIG 02-98 J. Clin. Oncol., 31, no. 7, 2013, p. 860.
12. ENDRES, L., UIVAROSAN, D., TIT, D.M., POP, O., BUNGAU S., BUNGAU C., Iran. J. Public Health, 47, no. 4, 2018, p. 606.
13. DENARDO, D.G., COUSSENS, L., Breast Cancer Res., 9, no. 4, 2007, p. 212.
14. SCHMIDT, M., BOHM, D., VON TORNE, C. et al., Cancer Res., 68, 2008, p. 5405.
15. DENEKERT, C., LOIBL, S., NOSKE, A. et al. J. Clin. Oncol., 28, no.1, 2010, p. 105.
16. WEST, N.R, MILNE, K., TRUONG, P.T., MACPHERSON, N., NELSON, B.H., WATSON, P.H., Breast Cancer Res., 13, no. 6, 2011, p. 126.
17. BRATU, O.G., MARCU R.D., SOCEA, B., NEAGU, T.P., DIACONU, C.C., SCARNECIU, I., TURCU, F.L., RADAVOI, G.D., BRATILA, E., BERCEANU, C., SPINU, A.D., Rev. Chim. (Bucharest), 69, no. 7, 2018, p. 1813.
18. ONO, M., TSUDA, H., SHIMIZU, C. et al. Breast Cancer Res. Treat., 132, 2012, p.793.
19. YAMAGUCHI, R., TANAKA, M., YANO, A. et al., Hum. Pathol., 43, 2012, p. 1688.
20. LEE, H.J., SEO, J.Y., Ahn, J.H., Ahn, S.H., Gong, G., J. Breast Cancer, 16, no.1, 2013, p. 32.
21. ADAMS, S., GRAY, R.J., DEMARIA, S. et al., J. Clin. Oncol., 32, 2014, p. 2999.
22. LOI, S., MICHELS, S., SALGADO, R., SIRTAINE, N., JOSE, V., FUMAGALLI, D., et al. Ann Oncol., 25, no. 8, 2014, p. 1544.
23. DIECI, M.V., MATHIEU, M.C., GUARNERI, V., CONTE, P., DELALOGO, S., ANDRE, F., et al., Ann. Oncol., 26, no 8, 2015, p. 1698.
24. DENEKERT, C., VON MINCKWITZ, G., BRASE, J.C., et al., J. Clin. Oncol., 33, 2014, p. 983.
25. URSULESCU, C.L., URSARU, M., CIOBANU, D., NEGRU, D., LUPASCU, C., Rev. Chim. (Bucharest), 68, no. 5, 2017, p. 1143.
26. PEREZ, E.A., THOMPSON, E.A., BALLMAN, K.V. et al., J. Clin. Oncol., 33, 2015, p. 701.
27. LEONG, P.P., MOHAMMAD, R., IBRAHIM, N., et al., Immunol. Lett., 102, 2006, p. 229.
28. MIYASHITA, M., SASANO, H., TAMAKI, K., et al., Breast Cancer Res. Treat., 148, 2014, p. 525.
29. BURSTEIN, H.J., LACCHETTI, C., ANDERSON, H., et al. J. Clin. Oncol., 34, 2016, p. 1689.
30. CIMINO-MATHEWS, A., Ye X, MEEKER, A., ARGANI, P., EMENS, L.A., Hum. Pathol., 44, 2013, p. 2055.

Manuscript received: 18.03.2018