Variation of the T Lymphocytes According to Treatment in Breast Cancer

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Breast cancer is a multifaceted disease whose varied phenotype recapitulates only partially the biological complexity. At present, there are new approaches to the diagnosis and treatment of this form of cancer, but research should also focus on identifying and implementing other individual prognostic factors, factors that may lead to improved clinical decision making with regard to the patient, in order to establish an individualized treatment.

Keywords: breast cancer, T-lymphocytes, chemotherapy, radiotherapy, hormonal therapy

Experimental part

Breast cancer is a multifaceted disease whose varied phenotype recapitulates only partially the biological complexity. Due to very high absolute levels of incidence and, inevitably, mortality, breast cancer is one of the major forms of both prevention and treatment and, not for the sake of scientific research. Numerous efforts have been made over time to improve the survival rate through early diagnosis and multiple (combined) therapies. At present, there are new approaches to the diagnosis and treatment of this form of cancer, but research should also focus on identifying and implementing other individual prognostic factors, factors that may lead to improved clinical decision making with regard to the patient, in order to establish an individualized treatment.

Immunoeading is a dynamic process that consists of immunosuppression and tumor progression. Tumor progression has 3 phases: elimination, equilibrium and escape. In the elimination and balance phases, cancer cells are attacked by the CD8+ T lymphocytes, while the tumor escape phase inhibits the CD8+ T lymphocytes.

In order to better understand the effects of the treatment on the adaptive immune system, peripheral blood samples were collected from 50 patients diagnosed and treated at the Bucharest Prof. Dr. Alexandru Trestiaoreanu Oncological Institute, during 2012-2018, to determine the influence of T lymphocytes on tumor progression as possible prognostic factors in relation to the clinical and pathological parameters and their response to the adjuvant / neoadjuvant, hormonal or radiotherapy treatment. Chemotherapy regimens were established according to the ESMO and NCCN guidelines.

The 50 patients included in the study underwent adjuvant cytostatic and neoadjuvant chemotherapy consisting of EC chemotherapy (Epirubicin 90 mg / m2 IV, Cyclophosphamide 600 mg / m2 IV) followed by Docetaxel 100 mg / m2, CMF type (Cyclophosphamide 600 mg / Methotrexate 40 mg / m2 IV, 5-Fluorouracil 600 mg / m2 IV followed by Docetaxel 100 mg / m2 IV administered every 21 days) or FEC chemotherapy (5-Fluorouracil 500 mg / m2 IV, Epirubicin 100 mg / IV, Cyclophosphamide 600 mg / m2 IV, administered every 21 days) followed by Docetaxel (100 mg / m2 IV, given every 21 days). Patients who had positive hormonal receptors followed hormone treatment (Tamoxifen or Anastrozole). For patients confirmed with Her2 in the IHC (7 patients), Trastuzumab (6 mg / kg IV every 21 days for 1 year) could be given. Of the total patients, 20 representing 35.71% performed radiotherapy. Table 1 presents the statistical correlation between age and lymphocite T values.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Mean CD4 +</th>
<th>Mean CD8 +</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50</td>
<td>27.23%</td>
<td>44.03%</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>28.58%</td>
<td>50.28%</td>
</tr>
</tbody>
</table>

Table 1. Statistical correlation between age and lymphocite T values.

The first evaluation was performed on a total of 15 patients with the following lymphocyte counts:

- For CD4 + T, the minimum value was 24.18%, the average value was 52.77%.
- For CD4 + T, the minimum value was 13.35%, the average value was 28.85%.
- For CD8 + T the minimum value was 8.08, the average value was 19.81.
- For the CD4 + / CD8 + ratio the average value was 1.61 (minimum 0.71 and maximum 4.65).

A second evaluation was performed on a number of 15 patients who had the following values:

- For CD3 + T, the minimum value was 22.99%, the maximum value was 68.44% and the average value was 50.28%.
- For CD4 + T the minimum value was 10.84%, the maximum value was 44.03% and the average value was 27.23%.
- For CD8 + T the minimum value was 8.85%, the maximum value was 32.05% and the average value was 19.56%.
- For the CD4 + / CD8 + ratio the mean value was 1.61 (minimum value 0.76 and maximum value 4.25).

For the evaluation we had a total of 4 patients who had the following values:

- For the CD4 + / CD8 + ratio, the mean value was 1.61 (minimum value 0.76 and maximum value 4.25).

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- For CD3 + T, the minimum value was 11.42%, the maximum value was 71.11% and the average value was 45.76%.
- For CD4 + T, the minimum value was 11.42%, the maximum value was 38.61% and the average value was 25.67%.
- For CD8 + T the minimum value was 9.21%, the maximum value was 29.55% and the average value was 17.15%.
- For the CD4 + / CD8 + ratio the average value was 1.75 (minimum value 0.84 and maximum 3.71).

Table 5 depicts the average values for patients with 2 evaluations.
For CD3 + T the mean value was 50.89%. For CD4 + T mean value was 28.04%.
For CD8 + T the mean value was 19.68%. For the CD4 + / CD8 + ratio, the mean value was 1.41.

Table 6 shows the statistical correlation between T lymphocyte evaluations.
The first and second evaluations are strongly correlated statistically positive (p < 0.05) (table 7).

<table>
<thead>
<tr>
<th>Lymphocyte T</th>
<th>CD3.1</th>
<th>CD4.1</th>
<th>CD8.1</th>
<th>DP.1</th>
<th>DN.1</th>
<th>CD4_CD8.1</th>
<th>Age</th>
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<tr>
<td>CD3.1</td>
<td>Correlation Coefficient</td>
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<td>.512**</td>
<td>.180</td>
<td>.182</td>
<td>-.097</td>
</tr>
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<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.000</td>
<td>.066</td>
<td>.063</td>
<td>.323</td>
<td>.031</td>
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<td>.078</td>
<td>-.073</td>
<td>.042</td>
<td>.348**</td>
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<td>Sig. (2-tailed)</td>
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<td>.427</td>
<td>.456</td>
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<td>.371</td>
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<td>50</td>
<td>50</td>
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<tr>
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<td>-.073</td>
<td>.119</td>
<td>1.000</td>
<td>.080</td>
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<td>Sig. (2-tailed)</td>
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<td>.436</td>
<td>.225</td>
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<tr>
<td>DN.1</td>
<td>Correlation Coefficient</td>
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<td>.042</td>
<td>.088</td>
<td>.080</td>
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<td>Sig. (2-tailed)</td>
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<td>Correlation Coefficient</td>
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<td>.348**</td>
<td>-.575**</td>
<td>-.162</td>
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<td></td>
<td>Sig. (2-tailed)</td>
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<td>.000</td>
<td>.099</td>
<td>.757</td>
<td>.085</td>
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<tr>
<td></td>
<td>N</td>
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<table>
<thead>
<tr>
<th>Lymphocyte T</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Dev.</th>
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<td>75.13</td>
<td>52.77</td>
<td>10.31</td>
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<td>CD4.1</td>
<td>13.35</td>
<td>42.30</td>
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<td>6.98</td>
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<td>8.08</td>
<td>30.84</td>
<td>19.81</td>
<td>6.30</td>
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<td>DP.1</td>
<td>.12</td>
<td>9.41</td>
<td>1.32</td>
<td>1.60</td>
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<td>DN.1</td>
<td>.60</td>
<td>8.12</td>
<td>2.86</td>
<td>1.89</td>
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<td>CD4_CD8.1</td>
<td>.71</td>
<td>4.65</td>
<td>1.61</td>
<td>0.79</td>
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Table 2
MAXIMUM AND MINIMUM VALUES OF ANALYZED T LYMPHOCYTES (I EVALUATION) (N=50)

<table>
<thead>
<tr>
<th>Lymphocyte T</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Dev.</th>
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<td>22.99</td>
<td>58.44</td>
<td>39.28</td>
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<td>CD4.2</td>
<td>10.84</td>
<td>44.03</td>
<td>27.23</td>
<td>7.79</td>
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<tr>
<td>CD8.2</td>
<td>8.83</td>
<td>32.05</td>
<td>19.56</td>
<td>7.70</td>
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<tr>
<td>DP.2</td>
<td>.22</td>
<td>2.29</td>
<td>1.02</td>
<td>0.78</td>
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<td>DN.1</td>
<td>.51</td>
<td>6.53</td>
<td>2.47</td>
<td>1.90</td>
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<tr>
<td>CD4_CD8.2</td>
<td>.76</td>
<td>4.25</td>
<td>1.61</td>
<td>0.51</td>
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</table>

Table 3
MAXIMUM AND MINIMUM VALUES OF ANALYZED T LYMPHOCYTES (II EVALUATION) (N=15)
Of all the patients, 34 are hormone-treated and the mean values for CD4+ T were 28.17%, for CD8+ T 20.66% and a value of 1.49.

In the group of patients undergoing radiotherapy, the mean values for CD4+ T were 31.55%, for CD8+ T it was 17.06% and the CD4/CD8 ratio was 2.5.

For the patients undergoing CHT + Transtuzumab treatment the mean value for CD4+ T was 23.78, for CD8+ T 16.73 and the ratio of 1.48 (table 10). Table 11 shows the results for the statistical correlation of T lymphocyte values in patients undergoing hormone and radiotherapy.

Table 4
MAXIMUM AND MINIMUM VALUES OF ANALYZED T LYMPHOCYTES (III EVALUATION)

<table>
<thead>
<tr>
<th>LYMPHOCYTE</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
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<tbody>
<tr>
<td>CD3</td>
<td>4</td>
<td>25.86</td>
<td>71.11</td>
<td>43.76</td>
<td>18.85</td>
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<td>CD4.3</td>
<td>4</td>
<td>11.42</td>
<td>38.81</td>
<td>25.67</td>
<td>12.35</td>
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<tr>
<td>CD8.3</td>
<td>4</td>
<td>9.21</td>
<td>29.55</td>
<td>17.13</td>
<td>8.76</td>
</tr>
<tr>
<td>DP.3</td>
<td>4</td>
<td>0.78</td>
<td>3.77</td>
<td>1.68</td>
<td>1.41</td>
</tr>
<tr>
<td>DN.3</td>
<td>4</td>
<td>1.37</td>
<td>2.05</td>
<td>1.87</td>
<td>0.34</td>
</tr>
<tr>
<td>CD4_CD8.3</td>
<td>4</td>
<td>0.84</td>
<td>3.71</td>
<td>1.75</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Table 5
AVERAGE VALUES FOR PATIENTS WITH 2 EVALUATIONS (N=15)

Table 6
STATISTICAL CORRELATION BETWEEN T LYMPHOCYTE EVALUATIONS

Table 7
CORRELATION BETWEEN EVALUATIONS

Table 8
MEAN T LYMPHOCYTE FOR PATIENTS UNDERGOING HORMONAL TREATMENT (N=34)
There is a statistically significant difference in the CD4 / CD8 ratio. The CD4 / CD8 ratio is increased in the group of patients undergoing radiotherapy. An additional study is needed in a larger group of patients.

For patients undergoing treatment with Transtuzumab and hormone therapy, the mean value for CD4 + T lymphocytes was 23.78, for CD8 + 16.73 and the ratio was 1.48. For patients undergoing radiotherapy, the mean value for CD4 + T lymphocytes was 31.55 for CD8 + 17.06 and the CD4 + / CD8 + ratio was 2.51.

Table 13 presents the statistical correlation of T lymphocyte values in patients receiving HT and RT treatment, and table 14 shows the mean T lymphocyte for patients undergoing treatment with Transtuzumab + HT and RT.

The higher CD4 + T and CD4 + / CD8 + ratio are observed in the group of patients undergoing radiotherapy, but statistically there are differences, but they are not statistically significant (p> 0.05).
et al. [15] in their study reported that the low CD4 / CD8 ratio can independently predict mortality from all causes. Shah et al., [7-8] which has prospectively confirmed that CD8 + T lymphocytes are more sensitive and more specific, also supported by the study of Mahmut Ozsahin et al. [6] and Mahmoud et al. [5] did not report significant differences between the CD8 + T lymphocytes, but they are not statistically significant (p>0.05).

Results and discussions

In breast cancer, the extensive tumor infiltration by cytotoxic CD8 T cells was strongly associated with patient survival and response to treatment. The presence of CD4 + T cells was associated with both good response to treatment and mitigation of the antitumor response [1-8].

Statistical analysis for the group of patients undergoing hormone therapy and the group of patients undergoing hormone therapy concluded that there are differences in CD4 + and CD8 + T lymphocytes, but they are not statistically significant (p>0.05).

For patients undergoing hormone therapy, the mean value for CD4 + T lymphocytes was 23.78, for CD8 + 16.73 and the ratio CD4 + / CD8 + of 1.48.

For patients undergoing hormone therapy, the mean value for CD8 + T lymphocytes was 28.17, for CD8 + 20.06 and the CD4 + / CD8 + ratio of 1.49.

Statistical analysis for the group of patients undergoing hormone therapy and the group of patients undergoing hormone therapy concluded that there are differences in CD4 + and CD8 + T lymphocytes, but they are not statistically significant (p>0.05).

Chemotherapy can enhance the immune response by improving the immune effector cells or by exhaustion of the immunosuppressive populations. In breast cancer, taxanes can enhance the function of NK and T cells according to Carson et al. 2004 [16], and the increase in the TIL percentage in the neoadjuvant context [23]. Docetaxel increases Th1-associated cytokine levels, while decreasing the inflammatory markers in metastatic disease, according to Tsavaris et al. 2002 [24]. Several studies have demonstrated the immunomodulatory properties of radiotherapy (RT). RT induces the death of the immunogenic cells (ICD), increases the MHC-I expression in both normal and cancer cells, stimulates the chemotaxis and recruitment of T cells and T cells into the tumor by inducing intracellular adhesion molecules, cytokines and chemokines and inducing CTL primacy [18-22]. The higher CD4 + T and CD4 + / CD8 + ratio was observed in the group of patients undergoing radiotherapy, but statistically there are differences, but they are not statistically significant (p> 0.05).

Conclusions

Although it has been considered that chemotherapy has immunosuppressive effects, contrary, it has also been shown to have immunomodulatory effects. The study demonstrated that the adaptive immune system is altered after chemotherapy for at least 9 months by assessing the CD4 + T lymphocytes, CD8 + T and the CD4 + / CD8 + ratio. Additional investigations will be needed to determine whether therapy should be modified to avoid the most serious effects on the immune system. Interestingly, for patients undergoing metastatic Capecitabine treatment, T cell antitumor reactivity was associated with lower values of all major subtypes of circulating lymphocytes (3-6 months) and prolonged (> 9 months) prolongation of CD4 + T cells. This is consistent with a smaller previous study showing a sustained decrease in the CD4 + T cells, but not CD8 +, after FEC breast cancer chemotherapy [3].

Conclusions

Although chemotherapy and hormone therapy can modulate and the immune system, e.g., letrozole in the neoadjuvant setting, reduces intratumoral FOX-P3 Tregs [29].
changes in the CD8+ and CD4+ ratios between the two evaluations.

Differences in hormonal treatment revealed that values increased after cytostatic treatment or radiotherapy. This observation suggests that hormone therapy helps in recovering lymphocyte populations after chemotherapy or radiotherapy. Hormone therapy also seems to help restore the T cell lymphocytes, thus the cellular immune response capacity, following the immune-induced immune suppression and chemotherapy. From analysis of the T lymphocyte percentages for radiotherapy patients, the mean CD4+ T was 31.55 for CD8+ 17.06 and the CD4+ / CD8+ ratio was 2.51.

References