THE IMPORTANCE OF IMMUNOLOGICAL MARKERS IN BREAST CANCER

MANUELA MIHALACHE, MARIA RADU, C. MIHALACHE

"Lucian Blaga" University of Sibiu

Abstract: *The biological markers are substances produced by the tumour, detectable in the periphery blood, urine, tumour tissue and may be identified through radio – immunology laboratory methods. The most usually biomarkers of breast neoplasms with prognostic and predictive importance for diagnosis and treatment are the estrogen and progesterone receptors, HER – 2 / neu and CA15-3, useful in monitoring the treatment and supervising the disease evolution.*

Keywords: *mammary carcinoma, biological markers*

Rezumat: *Markerii biologici sunt substanţe produse de tumoră, evidenţiabile în sângele periferic, urină, ţesut tumoral, identificabile prin mijloace de laborator radio-imunologice. Cei mai uzuali biomarkeri din neoplazia mamară cu rol prognostic şi predictiv pentru diagnostic şi tratament sunt receptorii de estrogen şi progesteron, HER-2/neu şi CA15-3, util în monitorizarea tratamentului şi urmărirea evoluţiei bolii.*

Cuvinte cheie: *cancer mamar, biomarkeri*

INTRODUCTION

Despite of the substantial progress of oncology recorded in the last decades, mammary neoplasm still remains a diagnosis of large severity, being on the first place in the hierarchy of cancers in women, with more than one million new cases yearly and more than 400.000 deaths (5,6). Prognostic gravity is related to the risk of recurrence and metastasis (11). The research of the last years showed a diversity of biological products – the biological markers that, on one hand are substances produced by the tumour itself and detectable in the periphery blood, urine, tumour tissue and, on the other hand, they are those biological phenomena intimately related to the presence of the tumour, which could be identified through laboratory methods, especially through the radio-immunological ones. The most important biomarkers of the mammary neoplasia with prognostic and predictive importance for the diagnosis and treatment are those that measure or are associated to biological processes involved in the tumour progression (size of proliferation, developing factors, genes suppression, oncongene alteration). Besides these, the status of the hormonal receptors at tumoral level plays a predictive part in the mammary carcinoma, regarding the response to the adjuvant treatment and metastasis. Currently, the estrogen and progesterone receptors, along with HER-2/neu represent indicators of the treatment and concerning the serous markers, CA15-3 is useful in monitoring the treatment and supervising the disease evolution.

PURPOSE AND RESEARCH METHOD

This paper belongs to breast cancer studies and aims at performing a preliminary, prospective analysis over an homogenous batch of 181 patients diagnosticated with mammary carcinoma, with a view to a clinical, therapeutic characterisation and to identify the theoretical possibilities for using the immunological markers in breast cancer diagnosis, as well as to the early detection of the disease recurrence, its use as prognostic factors and treatment monitoring.

MATERIAL AND METHOD

We assessed a series of clinical and para-clinical aspects, gathered from breast cancer patients (local and regional extension level of the tumour, the number of invaded ganglions, diseases stage, hormonal status, local recurrence moment and metastasis), with a view to emphasize certain possible correlations between them and the value of the immunological markers.

TARGETED MARKERS:

Hormonal receptors (estrogen - RE and – progesterone RP); antigen CA15-3 (VN < 20-40UI/l) and HER2/neu.

STUDY INCLUSION CRITERIA:

TNM stadialization; histological confirmation; information related to the tumoral extension level and ganglions invasion; patients that underwent the specific oncologic treatment; the value of the immunological markers at the beginning of the treatment and during the treatment; objective documentation of the relapse moment (metastasis or local and regional recurrence).

RESULTS AND DISCUSSIONS

After applying the evidenced selection criteria, a batch of 181 patients was shaped. Regarding the age of the patients, it was between 26 and 75 years old, the average age being 50,5 years. In terms of stadialization, according to TNM classification, the majority of cases – were classified in the III category, predominant hystopathologic aspect, (90%) being ductal invasive G2

carcinoma. The distribution per stages of the tumours taken into consideration: IA and IB – 5 patients; stage II IIA and IIB – 66 patients; stage IIIA and IIIB – 100 patients; stage IV – 10 patients.

Regarding the type of surgery, 11 radical mastectomies were performed and 60 sectorectomies with axillary lymphadenectomy; there were 10 patients who did not undergo any surgery.

Regarding the majority of the patients, CA15-3 was quantified at the beginning of the treatment, as well as at the end of the sequential therapy; it varied between 1,8U/ml and 305 U/ml; out of the removed tumoral formation, the hormonal receptors (estrogen and progesterone) were identified through immunohistochemical methods: 137 patients were identified with positive hormonal receptors, as well as with the Her2-new fraction: 55 patients had positive Her 2-new and 52 - negative; they were not detected in 74 patients.

CA15-3 (VN<30U/ml)**:**

Out of the total number of the patients, 46 (25,41%) patients registered CA15-3 over the normal limit and 135 (74,58%) patients had normal values of CA15-3 or, it has never been detected.

By correlating the value of CA15-3 at the moment of diagnosis to the disease stages, we observed that the advanced stages of the disease were associated to an increase value of CA15-3 in 39 patients (stage III 31 (17,1%) patients and stage IV – 8 (4,4%) patients); while the disease initial stages were associated to an increase value of CA15-3 in only 10 patients (stage I – 2 (1,1%) patients and stage II – 8 (4,4%) patients); significant data, statistically speaking. (Tabel.1)

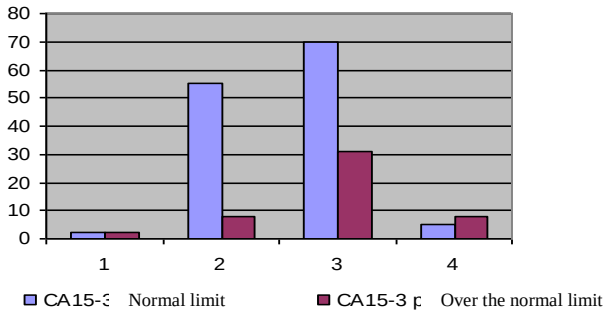
Table no. 1. Correlation of CA15-3 to the diseases stages

CA15-3	Stage I	Stage II	Stage III	Stage IV	Total
Normal limits	2 (1,1%)	55 (30,4%)	70 (38,7%)	5 (2,8%)	135 (74,58%)
Over the normal limit	2 (1,1%)	8 (4,4%)	31 (17,1%)	8 (4,4%)	46 (25,41%)
Total	4 (2,2%)	63 (34,8%)	101 (55,8%)	13 (7,2%)	181 (100%)

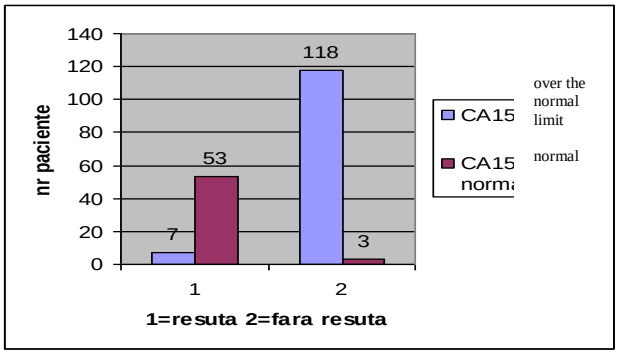
These results show that: CA15-3 identified at the moment of the diagnosis did not have any importance in establishing the diagnosis; yet, the value of the marker over the normal limit was more frequently associated to an advanced stage of the disease (out of 46 patients with CA15-3 over the normal value identified *on the moment of the diagnosis, 39 were in an advanced stage of the disease - stage III and IV), result statistically significant.*

Following ***the value of the CA 15-3 marker in evolution,*** it was observed that out of the total number of patients, 125 (69,06%) had a normal value of CA15-3 and 56 (30,9%) patients had an increased value of CA15-3 . Out of those 125 (69,06%) patients of normal CA15-3, 7 (3,8%) patients registered relapses, (there were 2 patients with recurrence and 5 patients with metastases); the rest of 118 (65,1%) patients did not record any relapse. Regarding those 56 (30,9%) patients with increased values of CA15-3, 53 (29,2%) patients registered relapses (8 patients with recurrence, 43 patients with metastases and 2 patients, both with recurrence and metastasis). There were 3 (1,65%) patients, who did not register any relapse (picture no. 2).

Picture no. 1. Correlation of CA15-3 at the moment of diagnosis between the stages of the disease. P=0,036*likelihood ratio



Picture no. 2. Correlation of CA15-3 in evolution with the moment of relapse

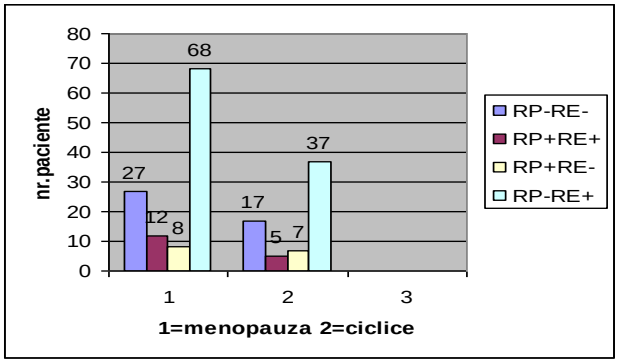


It was observed that a large number of patients, 29,2%, with relapse of the disease registered an increased value of CA15-3 in evolution. CA15-3 monitoring per stages after therapy brought precocious information regarding the disease relapses.

The hormonal receptors (RH-estrogen receptors - RE; progesterone receptors-RP): Out of the total number of the patients, 137 (75,69%) patients had at least one positive hormonal receptor and 44 (24,30%) patients had negative hormonal receptors. Regarding the studied batch, by correlating the presence of the hormonal receptors to the hormonal status (menopause / cyclic), it was observed that (Picture no. 3):

- 88 (54%) patients with *positive hormonal receptors* (RP+RE+;RP+RE-;RP-RE+) are *in menopause* (for RP+RE+ 6,6%; for RP+RE- 4,4%; for RP-RE+43%) and 49 (27,1%) patients with *positive hormonal receptors* (RP+RE+;RP+RE-;RP-RE+) are *cyclic* (for RP+RE+2,8%; RP+RE- 3,9%; RP-RE+20,4%)
- 27 (14,9%) patients with *negative hormonal receptors* are in menopause and 17 (9,4%) patients with *negative receptors* are *cyclic.*

Picture no. 3. Correlation of RH (hormonal receptor) to the hormonal status

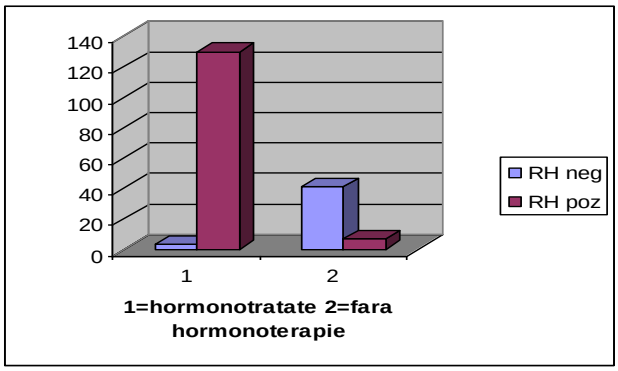


P=0,896 likelihood ratio

The presence of the positive hormonal receptors is higher in the case of the women patients in menopause; the result is not significant from the statistical point of view.

By correlating the RH to the hormonal treatment, we noticed that 130 (70,8%) patients with positive hormonal receptors (RP+RE+;RP+RE-;RPRE+) underwent hormonal treatment; and only 7 (3,9%) patients with positive hormonal receptors (RP+RE+;RP+RE-;RP-RE+) did not undergo any hormonal treatment. (Picture no. 4).

Picture no. 4. RH correlation to the hormonal treatment

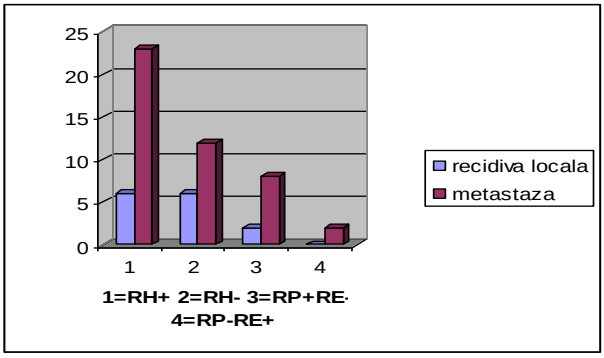


P=0,000 likelihood ratio**

*The presence of the hormonal receptors is a predictive factor of the hormonal therapeutic conduct; it is significantly associated to a proportion of 99%, p=0,000**.*

By correlating the RH to the occurrence of the disease relapse, we noticed that out of the studied patients, in 29 (49,1%) patients with positive hormonal receptors, the therapy failed, thus: 6 (20,6%) patients were recorded with local and regional recurrence and 23 (79,3%) patients with distance metastasis (p=0,32) and regarding 18 (30,5%) patients with negative hormonal receptors, the therapeutic failure was as follows: 6 (33,3%) patients with local and regional recurrence and 12 (66,6%) patients with distance metastasis (p=0,98) (Picture no. 5).

Picture no. 5.Correlation of RH to the relapse of the disease



The results were not significant statistically speaking. Still, the majority of the patients with relapse (16,94%) had positive progesterone hormones (RP +RE) and only 3,38% patients with relapse had positive hormonal receptors (RE +.RP –).

Her 2/new

Regarding the studied batch, 55 (30,3%) patients had positive Her2new; 52 (28,7%) patients had negative Her2new and in 74 (40,9%) patients, it was not detected.
By correlating the presence of Her 2/new to the ganglions invasion, it was noticed that 24,3% patients with ganglions invasion > 3 ganglions had positive Her2new and 6,1% patients with ganglions invasion < 3 ganglions had positive Her2new; (Table 2).

Table no. 2. Correlation of Her 2/new to the ganglions invasions

HER2new	Ggl<3	Ggl>3	Total
Negative	19 (10,5%)	33 (18,2%)	52 (28,7%)
Positive	11 (6,1%)	44 (24,3%)	55 (30,4%)
Unidentified	38 (21%)	36 (19,9%)	74 (40,9%)
Total	68 (37,6%)	113 (62,4%)	181 (100%)

P=0,001 likelihood ratio**

*So, the presence of Her2new may be considered a negative prognostic factor that may be associated with a precision of 99%, p=0,001** with the ganglions invasion >3 ganglions.*

By correlating Her2/new to the stage of the disease, it was noticed that: the advanced stages of the disease, such as stage III and IV are frequently associated to the presence of Her2new negative prognostic factor, in proportion of 17,7% and respectively of 6,1%; while regarding the disease stages I and II, 6% patients and respectively 6,1% patients had Her2new positive, significant data from the statistical point of view (p=0,001**)**. Her2new is a negative prognostic factor.**

It resulted that the majority of the patients with positive Her 2new (3+), that is 54 (29,8%), underwent specific treatment with Herceptine +/- cytostatics, significant data, statistically speaking (p=0,000**) (Table 4).

Her2new is a predictive factor of the adjuvant therapeutic conduct or in metastasis.

Table no. 3. Correlation of Her2/new to the specific treatment

HER2new	Herceptine	Without treatment with herceptine	Total
Negative	0	52 (28,7%)	52 (28,7%)
Positive	54 (29,8%)	1 (6%)	55 (30,4%)
Unidentified	0	74 (40,9%)	74 (40,9%)
Total	54 (29,8%)	127 (70,2%)	181 (100%)

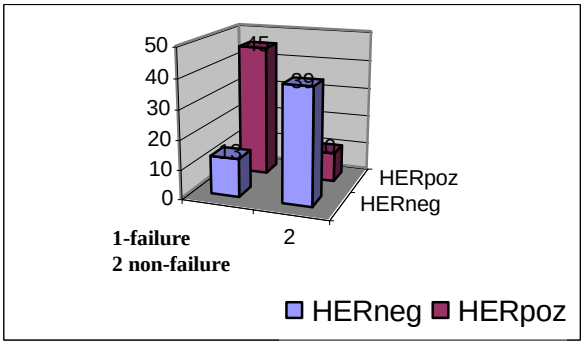
P=0,000 likelihood ratio**

Out of the total number of patients, in 45 (24,7%) patients with positive Her2new, relapses of the disease were recorded and the rest of 10 (5,5%) patients with positive Her2new were without relapse (Table 5 and picture no. 6).

Table no. 4. Correlation of Her2/new to the presence of disease relapse

H2Rnew	Local and regional recurrence	Metastasis	Recurrence and metastasis	Without relapse	Total
Negative	3 (1,65%)	9 (4,9%)	1 (0,5%)	39 (21,5%)	52 (28,7%)
Positive	7 (3,8%)	36 (19,8%)	2 (1,1%)	10 (5,52%)	55 (30,3%)
Unindentified	5 (2,7%)	1 (0,5%)	0	65 (35,9%)	74 (40,8%)
Total	15 (8,2%)	46 (25,4%)	3 (1,65%)	114 (62,9%)	181 (100%)

Picture no. 6. Correlation of Her2/new to the presence of relapse



Her2 new seems to be a negative prognostic factor in the evolution of the disease, being associated to the presence of relapse, a significant result, from the statistical point of view, p=0,01.

CONCLUSIONS

Breast cancer remains a disease with an infaust prognostic despite the present therapeutic aggressiveness (11). The main cause for the treatment failure in mammary carcinoma is represented by the process of metastasis favoured by the late diagnosis in many cases, by the insufficiency of the associating criteria for appreciating the biological behaviour of the tumour and by the impossibility for detecting the micro-metastasis in due time (1,2). The most important biomarkers of the mammary neoplasia with prognostic and predictive importance for diagnosis and treatment are estrogen and progesterone receptors, along with HER-2/neu as treatment indicators, while CA15-3 is useful in monitoring the treatment and in supervising the evolution of the disease.

The diagnostic value of CA15-3 depends on the disease prevalence, sensitivity and specificity. For the diagnosis, the results are correlated to the clinical and para-clinical parameters.

CA15-3 monitoring per stages after the treatment, brings about new precocious information regarding the disease recurrence (a few months before being clinically detectable).

The estrogen and progesterone receptors are established in all patients, both before and after the menopause, indicating which patient benefits from hormonal treatment.

Her2/new values play a part in choosing the adjuvant treatment with Trastuzumab (Herceptine) +/- treatment based on anthracyclines. The HER2/neu test is a predictive indicator for relapse (local recurrence or metastasis).

BIBLIOGRAPHY

1. Polyak K, "Breast Cancer Origins and Evolution J. Clin Invest 2007;
2. Foote FJ, Stewart F, "Lobular Carcinoma in situ Rate Form of Mammary Carcinoma Am J Pathol;
3. Schmitt S, Morrow M, "Lobular Carcinoma in situ Current Concepts and Controversies. Seminars in Diagnostic Pathology 1999;
4. Shing MM, Devi R, Walia I, Kumar R, "Breast self Examination for Early Detection of Breast Cancer. Indian Journal of Medical Sciences 1999;
5. Zhang Z F, Vassilaki M, "Essential Elements of Effective Cancer Screening Program; Screening for Breast and Cervical Cancer, Budapesta 2000;
6. Thurfjell EL, Lernevall KA, Taube AS, "Benefit of Independent Double Reading in a Population-based Mammography Screening Program Radiology 1994;
7. Feig SA, "Estimation of Currently Attainable Benefit from Mammographic Screening of Women Aged 40-49 years. Cancer 1995;
8. American Joint Committee on Cancer: Cancer Staging Manual Edition Springer New York 2002;
9. N. Ghilezan, A.C. Rancea, C. Vitoc, Gh. Peltecu "Cancerul mamar ghid de diagnostic si tratament", Radioterapie şi Oncologie Medicală, vol XII 2006 nr. 1;
10. Camizares F, Sola J, Perez M - Preoperator Values of CA15-3 and CEA as Prognostic Factors in Breast Cancer: a Multivariate Analysis. Tumor Biol, 2001 Sep-Oct; 22(5): 273-281;
11. Ebeling FC, Schmitt UM - Tumour Markers CEA and CA15-3 as Prognostic Factors in Breast Cancer - Univariate and Multivariate Analysis. Anticancer Res, 1999 Jul-Aug; 19(41): 2545-2550;