TABLE OF CONTENTS

Abbreviations list ........................................................................................................ 8

Introduction ................................................................................................................. 10

STAGE OF KNOWLEDGE

CH. I: Breast and Bone Histology ............................................................................. 11

CH. II: Natural History of Cancer ............................................................................. 24

CH. III: Breast Cancer ............................................................................................. 34

PARTEA SPECIALA

Objectives .................................................................................................................... 69

CAP. IV: Material and method.................................................................................. 70

CAP. V: Results .......................................................................................................... 85

CAP. VI: Discussions .................................................................................................. 191

CAP. VII: Conclusions ............................................................................................... 217

Bibliography ............................................................................................................... 219
INTRODUCTION

Being aside the neoplasm patient is the most difficult thing. There is a lot of pain and suffering. Mammary gland cancer has become a major health problem.

In the malign pathology of the woman in our country, breast cancer is the most frequent and its incidence is ever growing. In the last few years, together with the TNM system and pTNM, a lot of new ways of diagnosing and treating the neoplasma patient have appeared as a result of the new imagistic techniques, of the immunohistochemical techniques of detecting estrogenic and progesteronic hormone receivers and together with the factors of tumor proliferation and of the appearance of molecular targeted therapies. My clinic sense has given me the feeling that patients in the 4th degree of breast cancer with bone metastases would live longer and better than those with other localizations of the metastases of breast cancer. The suggestion of University Professor Dr. Florinel Badulescu met my preoccupations. I wanted to carry out everything that meant diagnosis in treating of 4th degree mammary gland cancer with bone metastases. The presence of bone metastases meant treatment with osteoclast inhibitors.

Key words: mammary gland, bone metastasis, osteoclast inhibitors, punction-biopsy, hormone receptors, HER2-Neu, molecular classification.
I. STAGE OF KNOWLEDGE

Chapter I – “Breast and bone histophysiology” describes classical elements of the anatomy, physiology and embryology of the mammary gland and of the bone, together with a description from the tissue level to the levels of cellular and individual components exemplifying the functions of each component, insisting upon the mechanisms that regulate the bone turn-over.

Chapter II - Entitled “The Natural History of Cancer” is dedicated to a brief but didactic presentation of the main events that appear in the natural history of cancer. Biology and molecular genetic studies have suggested the fact that the oncogenesis process follows three stages: initiation, promotion and malignant progression. These stages bring about numerous genetic modifications that progressively accumulate in the neoplastic cells, this phenomenon being a major feature of the malignant neoplasm.

Relying on up to date references in the domain, the chapter next describes the main mechanisms responsible for the appearance of osteolitic and osteocondensed metastases stressing the role of the bone micromedium during this stage of the development of a malignant tumor.

Chapter III - “Breast Cancer” focuses on epidemiology, genetics, diagnosis and staging (ranking), pathology, diagnosing bone metastases and treatment principles of the most frequent malignant tumor with women. About 10% of the human breast cancer cases can be directly connected to a mutation on the line of germinal cells. The mutations at the level p53, BRCA-1, BRCA-2<erb2 (HER-2/neu) genes are described turn by turn.
Establishing the stages of breast cancer is based on the TNM system and this is done according to a pretherapy examination which has to state precisely the anatomic expansion in the three compartments: Primary tumor (T), regional adenopathy (N) and metastases (M).

Tumoral lesions are the most important lesions of the breast, possibly having their origin in all anatomic structures: skin coverage, preglandular and retroglandular fat tissue, mesenchymal conjunctive tissue and the epithelium of the ductal lobular terminal unit (TDLU or UTDL). A histological classification of the mammary tumors includes:

1. **benign tumors**: adenomas, papiloms, papilomatis, ductal hiperplasy, benign mioepithelium neoplasms, fibroepithelial benign tumors, mesenchymal benign tumors;
2. **malignant tumors**: noninvasive (carcinoma in situ) and invasive (invasive ductal carcinoma, lobular invasive, coloid carcinoma, medular carcinoma); mesenchymal malignant tumor.

Next there is a presentation of the main diagnosis methods of bone metastases, then there are presented the symptoms and the signs of the bone metastases disease, as well as the necessary imagistic investigations (X-ray, scintigraphy, CT, RMN, PET-CT).

Breast cancer benefits from a complex oncologic therapy. The new tendencies indicate a “multimodal ” treatment adapted to each stage for a system disease.

The principles of surgery treatment, chemotherapy, radiotherapy, hormonotherapy, molecular targeted therapy, as well as the therapy algorithms for each stage of the disease are discussed with regard to the latest guides accepted in literature.
II. OWN CONTRIBUTIONS

Chapter IV - entitled “Material and Method” opens with the enumeration of the objectives of the study:

- Retrospective study of metastasis breast cancer in Valcea County between 1999-2010.
- Selective study of bone metastases at these patients during the time mentioned above, given the great frequency of these secondary determinations.
- Establishing a diagnosis, treatment, prognosis and monitoring pattern and treatment standard in the management of bone metastases breast cancer.

Next there are described the groups of patients used in the epidemiologic, histopathologic and statistic study.

There was identified a total number of 1,194 cases with mammary gland neoplasm during that time, out of which 712 presented secondary determinations (145 when diagnosed and 567 with the disease in progress)

In order to assure the accuracy of the study, there have been randomly selected 213 cases with secondary determinations, these being the subject of the detailed epidemiological clinic, histopathologic study. The groups for study were established in the following way:

A. The total of 1,194 cases were included in the global epidemiological study.

B. The 213 cases selected with metastases were included in a detailed study and were also devided into two subcategories:

b1. Patients who presented with metastases when diagnosed (stage IV), 35 in number.
b2. Patients who developed metastases during the study (178 patients)

The therapy strategies used in the study comprised:

A. Osteoclast inhibitors (IO): Clodronatum, Acidum clodronicum (Sindronat), Pamidronatum disodium (Aredia), Acidum ibandronicum (Bondronat), Acidum zoledronicum (Zometa).

B. Hormonotherapy
At premenopause, there was practised radiologic, pharmaceutical and surgical oophorectomy with Goserelin-Zoladex (f=3.6mg). At menopause there were administrated:
(i) Antiestrogens (Tamoxifen),
(ii) Aromatasa inhibitors (Aminoglubemid, Letrozol, Exemestan and Anastrozol),
Progestine (Medroxiprogesteron acetate, Megestrol acetate)

C. Chemothepany
The treatments with mono / polichemothepany were:
(i) Monochemothepany: Taxanes (Paclitaxel), Docetaxel), Antimetabolits (Capecitabina);
(ii) Polichemothepany: classical or modified CMF series; Schemes with antracicline (FEC,FAC,EC,AC); Schemes with taxanes and antracicline (EP,AP); Schemes with gemcitabin and carboplatin:CBP.

D. Molecular targeted therapy: (i) Trastuzumab: trial initial dose of 4mg/kgbody, PEV slowly for 90 minutes, then maintenance dose of 2mg/kg, weekly; (ii) Bevacizumab: 10mg/kg, PEV once every other week.

E. Radiotherapy: hormon suppression and palliative role.

F. Surgery was first of all used in the medular compression syndrome.

Histochemical and iminohistochemical methods contained the histopathological and immunohistochemical diagnosis.
Chapter V - “Results” presents in 5 points the main results of the work, as follows:

1. The Epidemiologic Study has shown that out of 10,868 recorded cancer cases, 1194 were breast cancers. The evolution of the patients with breast cancer was not significantly different from the cases of cervical or endometrium tumors, but much better than ovary tumors (Fig.1).

![Survival analysis depending on the localization of the tumor.](image)

**Fig 1.** Survival analysis depending on the localization of the tumor.

Survival analysing on stages showed a deacreasing order for stages 0, 1, 2, 3, and 4 (Fig. 2).

Out of the total number of 1,194 patients found with breast pathology, 145 already had metastases when diagnosed and 567 developed them later. 712 patients developed metastases during the study.
2. The clinical study showed a predominant localization of the primary breast tumor at the level of the left breast in our group-61,03%. There were further described in detail: (i) the debut syndrome, (ii) establishing stages cTNM, (iii) the diameters and features of the primary tumor, (iv) the frequency and number of regional limphoganglions, (v) the frequency and localization of the metastases with a focus on the bone metastases.

![Image](image_url)

**Fig2.** Survival curves for different stages of breast cancer

The 213 cases were identified as having: (i) bone metastases (B=114 cases); (ii) combined (C=71 cases); (iii) others (O=28 cases). The Kaplan Meier Curve highlights the highest survival probability for the group of patients with bone metastases, the explanation being the possibility of therapy with osteoclast inhibitors.
3. The **histopathological** study showed that the most frequent form of malignant breast tumor was invasive ductal carcinoma. Grading G2-G3 was the most frequent at the 213 detailed cases.

4. **Imunohistochemical study** of the primary tumor showed (Fig.3):
   - ER were expressed in 59.28% of the patients, and PR at 55.09%-
   - c-erbB-2 was over expressed at 17.36% of the patients.
   - EGFR was overexpressed in 16.17% of the patients, being associated with the advanced stages of the disease, with negative extrogenic receivers, this being a factor of unfavourable prognosis.
   - p53 was overexpressed for 28.14% of the patients.
   - Ki-67 was over expressed for 8.38% of the patients.

![Image of IHC on primary tumors]

**Figure3.** Examples of IHC on primary tumors.

Depending on the molecular subtype, Luminal A are the most frequent forms. The best survival appeared for Luminal B tumoral subtype (Fig.4).
5. The evolution under treatment for different stages of the disease showed an unfavourable evolution for the patients with other metastases than the bone, compared to those with bone metastases or combined metastases.

Figure 4. Survival depending on molecular classes.

Chapter VI - “Discussions” integrates the results of the study in the general problem of breast cancer pathology.

The epidemiological profile of the patients with breast cancer within the study group corresponds to the data in literature, to the relative frequencies met in other studies. The most frequent metastases were to the bone (341), then the combined ones (287). Other types of metastases were on the third place: lung, liver, others. Out of the total 712 cases which developed metastases, there were randomly selected 213 patients. These underwent a detailed epidemiological, clinical, paraclinical and therapy study.
Analysing our data, I have noticed that luminal A subtype was predominant (105 cases or 62.8%) being followed in frequency by the triple negative subtype (29 cases or 17.3%), luminal B (20 cases or 11.9%) and HER2 (13 cases or 7.8%). In literature too, subtype luminal A, with a growing expression of ER, is the dominant molecular form in breast cancer. It was also noticed that in luminal A, luminal B and Her2 types, bone metastases dominated, while for triple negative ones, bone metastases combined with other localizations dominated.

The overexpression c-erbB2 was identified in literature in 10-30% of the invasive breast carcinoma, and our study on patients with secondary disseminations, identified the overexpression at 17.36% of the patients probably reflecting the grown aggression of the tumors taken into account. In our study ER were expressed at 99 patients, 59.28% and PR at 92 patients, 55.09%; for 92 patients there were expressed both ER and PR. Global studies have shown an incidence of up to 70% of ER and PR positivity in breast cancer.

Secondary determinations at the bone level are very frequent, therefore they appear in approximately 70% of the patients with breast and prostate cancer and in approximately 15-30% of the patients with lung, gastro intestinal cancer, with cancer of the genital tract of the kidneys and of the urinary bladder. It seems that the bone represents the preferred target for metastases in women cancer and carcinoma are much more susceptible at secondary determinations at the bone level compared with sarcomas.

I have found proof of dissemination, at least at the lombar vertebrae and liver. The antomopathological analysis of the vertebrae metastases has revealed a molecular pattern HER2+ CK18+, but negative for ER, PR, CK5/6 and EGFR. In addition, these secondary tumors expressed E-caderin and BRCA1.
Conclusions

1. The bone metastases therapy in the case of the mammary gland cancer includes osteoclast inhibitors, hormonotherapy, depending on the status of estrogen and progesterone receivers chemotherapy depending on the presence or absence of visceral metastases and therapy with monoclonal antibodies in case the oncogene c-erbB-2 is present;

2. The osteoclast inhibitors (namely bisphosphants) also had a significant antitumor effect, preventing tumor proliferation;

3. Bone scintigraphy remains the standard exploration/investigation in supervising bone modifications, recommending the use of other imaging investigations, depending on the results.

4. The correct establishment of the initial stage of the disease (TNM) confronted with the post surgery and histopathological stages remain major elements for the evaluation necessary for establishing a standard therapy, but personalised for each patient as much as possible (“each patient has her own cancer”);

5. The compulsory characteristics for establishing a therapeutic approach, now considered as standard, are based on HER2-Neu, ER and PR statuses, as well as the ki-67 index;

6. As a result of the study there have been defined like in literature, the following molecular subtypes in this localization: luminal A, luminal B, HER2, triple negative or basal-like; their evolution under specific treatment corresponds to literature;

7. The establishment of standardized molecular subtypes requires biopsy-puncture with histopathological and immunopathological examination, puncture aspiration with cytological examination being reserved for limited situations
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Emergency County Hospital
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EDUCATION

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<td>“Nicolae Bălcesu” High school, Rm. Vâlcea,</td>
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<tr>
<td>Medical Doctor degree</td>
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<td>1977</td>
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PUBLICATIONS

Presented works/papers

Benign-malign, diagnosis significance and therapeutic attitude in peripheral adenopathy. Elena Pirici, A. Pirici, Maria Timofte, Cornelia Popescu, National Congress of Oncology, Bucharest, 1994


False allergic reaction/response to doxicicline. A. Pirtici, Elena Pirici, Ionica Pirici. Valcea medical days, Rm. Valcea, 12-14 January 2007


Morfopathology of basal cell carcinoma. A.Pirici, Elena Pirici, Ionica Pirici, N.D.Pirici. Valcea medical days, Rm. Valcea, 15-17 May 2009

Erythrodermia at the derivatives of carboxamide. A.Pirici, Elena Pirici, Ionica Pirici, N.D.Pirici. Valcea medical days, Rm. Valcea, 15-17 May 2009


Exanthema in current medical practice. A. Pirici, Elena Pirici, V. Pirici. Valcea medical Days, Rm. Valcea, 7-9 May 2010


Published papers


Ductal invasive mammary carcinoma–clinicopathological prognostic factors related to immunohistochemical expression of hormonal receptors and Her2/neu oncoprotein. Felicia Recăreanu, Cristiana Simionescu, Claudia V. Georgescu, Elena Pirici. Rom J Morphol Embryol. 52(3 Suppl):1059-64; 2011– ISI indexed journal, FI=0.4