THE RELEVANCE OF CYTOLOGICAL DIAGNOSTIC IN THE MAMMARY GLAND CANCER

E. Anton¹,³, E. Ancuta³, B. Doroftei¹,³, N. Ioanid⁴, Carmen Anton²,⁵*

University of Medicine and Pharmacy “Grigore T. Popa” - Iasi
1. Faculty of Medicine
2. Department of Mother and Child Medicine
3. “Cuza-Vodă” Obstetrics and Gynecology Clinical Hospital, Iasi
4. Regional Institute of Oncology, Iasi
5. “Sf. Spiridon” County Clinical Emergency Hospital, Iasi
*Corresponding author. E-mail: carmen.anton@umfiasi.ro

THE RELEVANCE OF CYTOLOGICAL DIAGNOSTIC IN THE MAMMARY GLAND CANCER (Abstract): Breast cancer is the second leading cause of cancer death in women, while in Eastern Europe the most common form of diagnosed cancer. Out of the multiple possibilities of early detection of mammary neoplasia that have been elaborated, only mammography has proved to be a simple, efficient method and of a high sensitivity, almost 90%. However, the cytological confirmation of diagnosis allows us to perform the preoperative radiotherapy treatment or poly chemotherapy.

Material and methods: we analyzed the informative value of these diagnosis methods in stage I mammary gland cancer (MGC). In this way, in the present paper we demonstrated that collecting samples through fine-needle aspiration biopsy allows the cytological confirmation of the diagnosis of stage I MGC in 30.7% cases.

Results and discussion: In stage I MGC young patients, under 35 years, the cytological confirmation rate is 22.2% and is lower as compared to the cytological confirmation rate in patients older than 35 years which is 37.9%. Also, for a tumor diameter < 0.5 cm, the prevalence of cytological confirmation was only 10.3%, while for the diameter of 0.6 – 1.0 cm the cytological confirmation was around 40.0%. Therefore, in order to improve the cytological diagnosis confirmation rate the tumor biopsy through the USG of the mammary glands is required. Moreover, the cytological investigation of the smear obtained by the first and second puncture was instrumental in confirming the diagnosis in 41.3% and 17.4% cases; the subsequent repetition of the punctures was not useful as it helped to confirmation of the diagnosis only in 9.3% cases. The frequency of diagnosis cytological confirmation depends on the tumor histopathological form and type of growth. Conclusions: Thus, the lowest prevalence was in the mixed forms - 12.5% cases, lobular cancer - 24.4% cases, while regarding the type of growth, for the rare forms the cytological confirmation rate was 7.7% and 31.5% cases for the schiros growth type. Keywords: MAMMARY GLAND CANCER, CYTOLOGY.

Breast cancer is the most common oncologic disease in women and is one of the major public health issues. Worldwide, breast cancer is the second leading cause of cancer death in women, while in Eastern Europe the most common form of diagnosed cancer. Among the most frequent involved factors in the outset of breast
cancer are included: the hereditary factor - genetically breast cancer develops in 3-5 cases; the genetic factors BRCA 1 and BRCA 2, should be more carefully studied, which up to now have been confirmed as having the greatest impact in breast cancer development risk, age over 35 years, hormonal contraception, incorrect nutrition, therapy with hormonal preparations, ionizing radiation, psychosocial factors and mental stress (1).

The questionable risk factors that are presented in various studies are very different and numerous, in some authors being citing over 300 in number, but none established with certainty. Immune factors along neuroendocrine factors are regarded as responsible for the clinical development of breast cancer (2).

Cancer research is a priority in all the laboratories of the world, in terms of uncovering the appearance causes of the malignant process, understanding the mechanisms of development, but most of all, the discovery of early diagnostic methods and effective treatment. Ignorance, fear of diagnosis, lack of health education and of efficient programs for prevention and screening, from our country cause diagnosis of the disease to be detected in the majority of cases in advanced stages, when treatment remains only palliative and very costly, in this cases the patient's suffering being immense (3).

Out of the multiple possibilities of early detection of mammary neoplasia that have been elaborated, only mammography has proved to be a simple and efficient method of a high sensitivity (almost 90%). In the same time, if the clinical examination is performed by an experienced physician, mammography can play an important role in screening. Moreover, the clinical diagnosis of palpable tumors usually does not present any difficulties because the clinical symptoms that indicate suspicion or clinical diagnosis of MGC are well-known (4).

Morphological factors of prognosis include the following: presence of tumoral emboli of the lymphatic and blood vessels of both tumor and adjacent mammary glandular tissue. The presence of tumoral emboli in the blood and lymph vessels was noticed in 14-29.5% MGC patients, who progressed after surgical treatment (5).

According to MoHeenico B. M., the most important criteria to assess the prognosis of MGC are: 1. The diameter of the tumor. 2. The degree of differentiation of the tumor after Bloom-Richardson. 3. The status of the regional lymph nodes. 4. The degree of expression of the steroid hormones receptors (RE, RP). 5. RNM synthesis activity markers: marking indices, the S-phase fraction, thymidine kinase activity, - Ki-67. 6. Growth factors receptors, including the oncogenes: epidermal growth factor receptor (EGF-R), HER2/neu, insulin-like growth factor receptors (IGF-R), somatostatin receptors. 7. Tumor suppressor genes - p53, -Nm 23 (6).

Also, due to the successes of molecular biology in the arsenal of researchers and clinicians there is a number of accurate indices which contributing to the assessment of predicting the efficacy of the MGC treatment and prognosis and selecting adjuvant and neoadjuvant therapy tactics in combined and complex MGC treatment. The problem concerning the researches in this field is to determine the set of informative indices, which would complement one another, which would enable at a minimum possible examination price to ensure the selection of the most efficient method in the treatment of each patient. The research spectrum in each particular case can depend on the progress stage, the age and
The relevance of cytological diagnostic in the mammary gland cancer

Concomitant pathology of the patient, planned therapy and material technical basis of the institution.

In this way, it is generally accepted that the actual methods of MGC treatment which include the surgical component, radiotherapy, chemotherapy (CT) and hormone therapy (HT) could ensure a 90 - 95% rate of over 5 years’ survival in stage I of MGC (7).

Still, the cytological confirmation of diagnosis allows us to perform the preoperative radiotherapy treatment or poly chemotherapy; which is why we analyzed the informative value of these diagnosis methods in stage I MGC.

Therefore, even with the mammographic or ultrasonographic clinical conclusion or assessment on the malignant character of the pathological process in the mammary gland, the neoadjuvant treatment becomes feasible only when the diagnosis is confirmed by one of the morpho-pathological methods, such as the cytological or histopathological method. According to the data in the international literature the cytological confirmation in the tumors with dimensions of less than 2.0 cm is problematic and does not exceed 20-25% cases as opposed to the extended malignant processes (8).

MATERIALS AND METHODS

The material of this study includes clinical and laboratory data analysis, the informational value of various investigation and treatment methods on 680 patients, selected between 1996 and 2004.

The patients included in the study were divided into three groups: the retrospective group, the prospective group and the control group.

In this way, the retrospective group included 496 stages I MGC patients, diagnosed and treated during 1996-2004. The retrospective group was divided into 2 groups according to the type of clinical development of tumoral process after treatment: the 1st group including 419 patients without progress for 5 years after the treatment, and the 2nd group including 77 patients with the progressing tumoral process after treatment.

On the other side, the prospective group included 66 stages I MGC patients, diagnosed and treated between 2007 and 2008. For the patients in the prospective group there were assessed the molecular expressions of the HER2/neu molecular markers, CEA tumoral markers, CA-15.3, hormonal receptors RE and RP, hormonal homeostasis and immunological indices.

Finally, the control group comprised of 118 patients, from which 56 patients with Ha, Ilb, or lila stage MGC, and 62 patients with benign diseases.

In the control group the immunological and homeostasis hormonal parameters were studied for comparison with the stage I MGC.

A special inquiry was developed for this study, which included 150 parameters regarding special body or tumor features, the informational of various methods of investigation and the results of different treatment variants applied.

Data analysis

The decisive prognostic factors determination was performed by discriminate analysis. By applying discriminate analysis - within the groups of the retrospective lot the decisive prognostic factors of the clinical evolution variance were determined in the stage I MGC patients.

RESULTS

In this way, the cytological investigation conducted on 475 patients confirmed the diagnosis of cancer of the mammary gland
only in 146 patients, which represented 30.7% cases. The cytological confirmation correlates with the age of the stage I mammary gland cancer patients (tab. I). As a matter a fact, as shown in the table, the diagnosis of MGC was cytological confirmed in 22.2% cases for the patients up to 35 years; in 24.1% cases for the 35-49 years’ patients - and in 37.9% cases for the 50-year-old and older patients (tab. I).

### TABLE I

The accuracy of cytological confirmation of the malignant process in stage I breast cancer patients by age.

<table>
<thead>
<tr>
<th>No</th>
<th>Patients Age</th>
<th>No. of recorded patients</th>
<th>Cytological confirmed diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. abs.</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 35 years</td>
<td>27</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>35-49 years</td>
<td>216</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 50 years</td>
<td>232</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>Total:</td>
<td>475</td>
<td>146</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 11.06, p < 0.05 \]

Also, the cytological conclusion variants were: cancer - 30.7%, the cancer suspicion conclusion -18.9% cases, proliferative process with atypical - in 19.4% cases.

For the other cases other variants of cytological diagnosis, without suspicion of cancer, have been established.

Of these the cytological conclusion for 1 in each 3 patients, which represents 17.5% - consisted in normal glandular epithelial cells without proliferation or atypical signs (tab. II).

### TABLE II

Cytological conclusion variants in stage I MGC versions by the number of conducted aspiration biopsies

<table>
<thead>
<tr>
<th>No.</th>
<th>Cytological analyses conclusion</th>
<th>Quantity of conducted aspiration biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>1</td>
<td>Cancer</td>
<td>117</td>
</tr>
<tr>
<td>2</td>
<td>Suspicion of cancer</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>Proliferation with</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>Glandular epithelial cells</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>Lipids, erythrocytes,</td>
<td>64</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 38.37, p < 0.01 \]

In addition, as it can be seen in table II, the results for the cytological confirmations by number of conducted aspiration biopsies were: the first biopsy was cytological confirmed in 41.3% cases, for the second biopsy the cytological investigation of the smear from the samples allowed the diagnosis cytological confirmation in 17.4% cases, while the aspiration biopsies repeated 3-4 times cytological confirmed the diagnosis only in 9.3% cases.

Also, we have to mention that the correct cytological conclusion prevalence analysis depending on the tumor diameter revealed that for a tumor diameter < 0.5 cm the correct conclusion of MGC was at-
The relevance of cytological diagnostic in the mammary gland cancer

tained only in 10.3% cases, for 0.6 – 1.0 cm in 40% and for 1.1 – 2.0 cm – in 34.3% cases (tab. III).

Moreover, the diagnosis cytological confirmation frequency depends on the histopathological form and type of tumor growth. 31.6% cases were cytological confirmed for the ductal form, for the lobular form 24.4% cases were cytological confirmed, for the mixed forms (ductal and lobular) 12.5% cases, and for the rare forms that unite a string of highly differen-
tiated forms of MGC (tubular, papillary, medullar, mucinous and other.) - in 41.7% cases (tab. IV).

In addition, the prevalence of the cytological confirmation is depending on the morphological type of the tumor: for the solid growth MGC type, 66.1% of the cases were cytological confirmed; for the schiros growth type 37.5% were cytological confirmed, while the lowest frequency rate of cytological confirmation was in the mixed forms - 7.7% cases (tab. V).

**TABLE III**

The frequency of the cytological confirmation for the malignant process in patients with stage I MGC by size of the tumor

<table>
<thead>
<tr>
<th>No.</th>
<th>Size of tumor</th>
<th>No. of marked patients</th>
<th>Cytological confirmed diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 0.5cm</td>
<td>39</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>0.6-1.0cm</td>
<td>160</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>1.1-2.0cm</td>
<td>213</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>Total</td>
<td>412</td>
<td>141</td>
</tr>
</tbody>
</table>

χ² = 35.0, p > 0.05

**TABLE IV**

Frequency of cytological confirmation in patients with stage I MGC by tumor morphological variant

<table>
<thead>
<tr>
<th>No.</th>
<th>Morph pathological variants</th>
<th>No of patients</th>
<th>Cytological confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ductal</td>
<td>345</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>Lobular</td>
<td>86</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>Ductal and lobular</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Rare forms</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>Total</td>
<td>475</td>
<td>146</td>
</tr>
</tbody>
</table>

χ² = 65.0, p > 0.05

**TABLE V**

Frequency of cytological confirmation in patients with stage I MGC by tumor morphological type.

<table>
<thead>
<tr>
<th>No.</th>
<th>Morphological type</th>
<th>No. of Patients</th>
<th>Cytological confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Solid</td>
<td>59</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>Schiros</td>
<td>56</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Rare forms</td>
<td>260</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>Unknown</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Total</td>
<td>475</td>
<td>146</td>
</tr>
</tbody>
</table>

χ² = 5.0 p > 0.05
It must be also mentioned that although the cytological diagnosis was confirmed only in 146 patients who represented 30.7% of cases, for the other 329 patients, 69.3%, the morph pathological diagnosis was determined by intraoperative extemporaneous histological investigations.

**DISCUSSION**

In this way, as previously mentioned, it is generally accepted that the actual methods of MGC treatment which include the surgical component, radiotherapy, chemotherapy (CT) and hormone therapy (HT) could ensure a 90 - 95% rate of over 5 years’ survival in stage I of MGC (7). Still, the cytological confirmation of diagnosis is allowing us to perform the preoperative radiotherapy treatment or poly chemotherapy; which is why we analyzed the informative value of these diagnosis methods in stage I MGC.

Therefore, although with the mammographic or ultrasonographic clinical assessment on the malignant character of the pathological process in the mammary gland being available, the neoadjuvant treatment becomes feasible only when the diagnosis is confirmed by one of the morpho-pathological methods, such as the cytological or histopathological method. According to the data in the international literature, the cytological confirmation in the tumors with dimensions of less than 2.0 cm is problematic and does not exceed a rate of 20-25% cases as opposed to the extended malignant processes (8).

Moreover, serum tumoral markers are used to assess mammary neoplasia evolution: the carcinoembryonic antigen (CEA) and the CA 15.3 (cancer antigen 15.3) (9). In this way, the CA 15.3 concentration occurs in women with the diagnosis of breast cancer, and its usefulness lies in evaluating the evolution of the illness, response to treatment and relapse assessment. The CA 15.3 tumor marker is a mucin-like antigen, with high molecular weight, issued primarily by cells of mammary glandular carcinoma. CA 15.3 is defined by its reactions with two monoclonal antibodies: one of them (11D8) binds to an antigen from human milk; the other (DF3) bounds to a membrane fraction of the carcinomatous cells.

The CA 15.3 blood titer increased in patients with advanced process, drops after chemotherapy treatment and rises again in the case of relapses or metastasis (in particular bone metastasis). Although the sensitivity of this marker in detecting early stages is reduced, lately there is an increasing tendency towards the idea of introducing it as a method of screening in the female population in the age group 45-54 years old (pre-and postmenopausal), considered to be the age group most prone to breast cancer. Also, determining molecular markers is an important tool for the diagnosis, treatment, assessment of therapy response. A slight physiological increase of the CA 15.3 antigen is registered in 4-7% of the nursing women and in about 8% of the pregnant women (9).

Moreover, carcinoembryonic antigen (CEA) is a specific marker for the digestive tract cancers (colorectal, stomach, pancreas, liver, biliary vesicle); subsequently, clinical studies have shown an increase in other malignant diseases (lung, mammary gland, ovary, cervix, prostate).

Historically speaking, the carcinoembryonic antigen was first described in 1965 by Gold and Freedman in a patient
with colorectal carcinoma. The carcinoembryonic antigen is a glycoprotein with the molecular weight of 220 kDa containing approximately 40% proteins and 60% carbohydrates. In this way, it is one of the onco fetal products during embryonic life and fetal development. Its production is suppressed after birth, reaching very low levels into adulthood, less than 3 ng/ml in non-smokers, less than 5 ng/ml in smokers. Immunohistological, the carcino embryonic antigen can be determined in elevated concentrations in the fetal gastrointestinal tract and pancreas, as well as in colorectal adenocarcinoma, and in lower concentrations in the normal intestinal tissue, intestinal mucosa, exocrine pancreas, and in the liver. The function of antigen carcino embrionar is not fully known; it is assumed that the CEA bound to the cellular membrane serves as mechanism for intracellular recognition functioning as a receptor. In addition, CEA is also assigned an immunosuppressive effect through the induction of macrophages and suppressor T lymphocytes (10).

Also historically speaking, we could also mention the unique research carried out in 1962 by H. Bloom, W. Richardson, E. Harries. The authors were able to trace the fate of 250 untreated patients mainly in stage III-IV MGC between the years 1805 and 1933: the median survival was 2.7 years, and the last patient died in the 19th year of surveillance without any treatment (2).

Of particular relevance in this context is also the fact that the prediction factors mirror the relations of reciprocity between tumor biology and treatment (for example, RE, RP and hormonotherapy; HER2/neu and herceptin therapy) (11). In fact, the prediction factors may also have prognosis significance. In this way, MGC is considered to be a heterogeneous pathology, with clinical evolution ranging in various patients from severe aggressiveness to the relatively benign.

**CONCLUSIONS**

MGC represents a polymorphic and pathogenic disease and it cannot be admitted that all subgroups of patients will obtain identical results from one tactic of treatment determined for all the patients with MGC. Thus, the concept of MGC both clinical and pathomorphological, combines different cell clones depending on its microstructure and biology. As a result, the evolution of the disease, the prognosis and the effectiveness of the treatment may vary in different patients at the same stage, depending on the degree of malignancy of the tumor, its histopathological structure, the degree of expression of molecular markers identification and also immunoresistance.

Also, some numbers-related conclusions could be represented by the fact that: collecting samples through fine-needle aspiration biopsy allows the cytological confirmation of the diagnosis of stage I MGC in 30.7% cases. In stage I MGC young patients, under 35 years, the cytological confirmation rate is 22.2% and is lower as compared to the cytological confirmation rate in patients older than 35 years which is 37.9%. For a tumor diameter < 0.5 cm, the prevalence of cytological confirmation was only 10.3%, while for the diameter of 0.6 – 1.0 cm the cytological confirmation was around 40.0%.

Therefore, in order to improve the cytological diagnosis confirmation, rate the tumor biopsy through the USG of the mammary glands is required.

The cytological investigation of the smear obtained by the first and second
puncture was instrumental in confirming the diagnosis in 41.3% and 17.4% cases; the subsequent repetition of the punctures was not useful as it helped to confirmation of the diagnosis only in 9.3% cases. Moreover, the frequency of diagnosis cytological confirmation depends on the tumor histopathological form and type of growth. Thus, the lowest prevalence was in the mixed forms - 12.5% cases, lobular cancer - 24.4% cases, while regarding the type of growth, for the rare forms the cytological confirmation rate was 7.7% and 31.5% cases for the schiros growth type.

REFERENCES