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RESEARCH ARTICLE

Cytological Diagnosis of Ovarian Tumors

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Abstract

Ovarian cancer is one of the most common oncology disorders of the female reproductive system. The aim of the study is to estimate the efficiency of cytological methods in identification of specific characteristics of ovarian cancer and other cancers of female reproductive system. The research was conducted in oncology clinics of Moscow (Russian Federation) and Sofia (Bulgaria) within a period between 2012 and 2019. The study involved 373 cancer female patients who were divided into two groups: the study group (242 women, average age 38.5 ± 7.8 years) and the comparison group (131 women, average age 42.1 ± 9.0 years). The study group included patients diagnosed with ovarian, breast, and endometrial cancer, while the comparison group comprised patients with stomach and lung cancer. The methods of light microscopy, immunocytochemistry and cytological methods were applied in this research. Two types of breast cancer (with ductal breast cancer predomination over the lobular one) and three types of ovarian cancer were specified. The final diagnosis of small cell lung cancer was 15% of lung cancer cases. This cytocentrifuge method was applied in 20% of ovarian cancer cases. Immunocytochemistry revealed a significant response to epithelial antibodies and a negative response to mesothelial ones in breast cancer ($p \leq 0.001$). Also, there was no response to monoclonal antigens in ovarian cancer ($p \leq 0.001$). This enabled the researchers to recognize and diagnose the mentioned cancer types. A one-year mortality of breast and ovarian cancer was established in 45-47% of patients, which indicated that disease was diagnosed at advanced stages. The study showed the cytological methods to be not equally used in different cancer types: mostly used in breast and ovarian cancers of the study group and almost not applied in lung cancer. In the comparison group, such results were obtained only in stomach cancer. Cell vacuolization was detected in 65% of ovarian cancer cases, while in 80% of ductal breast cancer cases there were specified clusters of spherical cells. Cell structures in the form of bands and chains were found in 75% of lobular breast cancer cases. Single signet ring cells were identified in 20% of stomach cancer cases in the comparison group. The results of the study proved the cytological methods to be significantly important in facilitating the diagnosis of adenogenous ovarian and breast cancer in the study group.

Keywords: *Ovarian cancer, Breast cancer, Endometrium, cytological methods, Monoclonal antigens.*

Introduction

In the Russian Federation, as well as in other countries of the world, the frequency of ovarian cancer cases is constantly increasing [1]. Thus, in 2010, ovarian cancer was diagnosed at average in 15 out of 100 thousand women, i.e., 0.015% of the population. This cancer type ranks third in terms of incidence among cancers of female reproductive system [2]. At the same time, ovarian cancer ranks first in terms of mortality [3]. This tendency is observed all over the world, which proves the problem acute. The reason of high mortality rates is conditioned by fact that ovarian cancer is usually diagnosed only at advanced stages (third or fourth) [4]. Therefore, the challenges in the diagnosis of ovarian cancer exist, which include the timely diagnosed disease, accurate detection of tumor site, and the nosology nature [5]. Thus, there is a need for morphological studies, as they specify the after-treatment management in many respects [6].

But this method is fraught with difficulties, since the complex and multifunctional structure of ovaries specifies a variety of possible primary tumor sources, such as surface and fallopian tube epithelium, ovums, granulosa cells, and chyle cells, which actually make up the typical structure of ovaries [7, 8]. Another tumor sources within the ovaries are accessory fallopian tubes, ovarian medullas, parovarium, paroophoron, as well as rete ovarii. The mesothelium can also be affected by tumors [9].

At least 150 different types of ovarian cancer are documented. The most common neoplasms are surface epithelial malignant tumors, epithelial-stromal tumors, secondary tumors and others [10]. Tumors can be either ovary specific or not and often of undetermined origin [11]. The mentioned histological method continues to be a standard technique in diagnostic assessment but due to the complexity of tumor differentiation, a relatively new technique has been recently in use -a cytological method [12].

It allows not only to determine the nature of the neoplasm (malignant or benign) but also to specify the histological type of cancer and to estimate the tumor extent. The cytological method is used both in medical examination in clinics and during operations [13]. The materials used for diagnosis in the cytological method are quite diverse: ascitic and pleural fluids, as well as puncture biopsy materials of the ovaries and lymph nodes. Moreover, peritoneal lavage, as well as rectal irrigation and, if possible, the materials obtained from the tumor itself are used [14].

Presently, cytological methods can be divided into two types depending on the analysis period. The first one includes preoperative procedures [15]. As a rule, that is a biopsy of the neoplasm, as well as investigation of ascitic and pleural fluids, and a biopsy of the posterior vaginal fornix. All these procedures are performed by means of aspiration puncture, using special thin needles.

The second type is postoperative, including tumor imprint or scraping, and peritoneal lavage. The researchers do not arrive at a general idea concerning the reasonable use of aspiration puncture. Some of them suggest that while performing the puncture, the cancer cells can be introduced into the intraperitoneal space, for example [16]. Others point to the lack of any real data on this issue [17]. Still, the sensitivity of the aspiration method reaches almost 100%, and has a slightly lower quality (about 90%) in diagnostic specificity and informative value [18]. It should be noted that the most informative material is usually obtained via the posterior vaginal fornix puncture.

Another distinguishing characteristic of aspiration method is its great suitability for young patients who want to maintain fertility. In younger patients (children and adolescents) neoplasms are typically similar to ovarian tumors and it is almost impossible to recognize them before the surgery.

Therefore, aspiration puncture for cytological analysis makes the surgery optional for the mentioned age group of patients [19]. The disadvantages of the aspiration method include the low informative value of the obtained material on condition of low aspirate cellularity or in some types of neoplasms, for example, borderline ovarian tumors and highly differentiated carcinomas [20].

Actually, while analyzing the ovary cellular material, cytological methods enable clinicians to specify differential characteristics of a cancerous tumor, namely the occurrence of glandular and papillary structures as well as cells with large vacuoles diffused in the cytoplasm [21]. Another diagnostic property is detection of certain cells with cilia at the apical end [22]. This sign is particularly distinctive in ovarian cancer.

The so-called psammoma bodies (calcified sloughed clumps) can also be a supportive mark in diagnosing the ovarian cancer. These signs are meant to be diagnostic ones in specifying the primary ovarian tumor site. If the diagnostic signs are absent, it is very difficult to perform a diagnostic assessment. For this, the immunocytochemical method is used [23]. It is based on the release of various antigens (Ber-EP4; CK 7, WT-1, CA-125, CA-19.9 and more) to estrogen receptors (serous tumors) and progesterone (endometrioid tumors).

This method is highly sensitive and provides an opportunity to specify the presence of a neoplasm as well as to determine the tumor process degree and its differentiation [24].

Other informative cytological methods for ovarian cancer diagnosis include the detection of cell proliferation markers, namely Ki-67 and proliferating cell nuclear antigens (PCNA). Meanwhile, the number of proliferative cells in the malignant and benign tumors can vary significantly: 33.0 ± 16.0 against 3.0 ± 3.0, respectively [25].Finally, according to the International Federation of Gynaecology and Obstetrics, cytological examination of ascitic and perinatal fluids as well as of peritoneal lavage is an essential condition for surgical intervention [26].

Such analyses as imprint cytology of the tumor, peritoneum, omentum, vaginal vaults and sites of the tumor invasion to nearby organs allow specifying the condition, its extent, and the histological type of the tumor. As a result, it enables clinicians to decide on the surgical intervention and make predictions. Due to the diagnostic challenge of the histological analysis, these are the cytological findings, which can provide the correct diagnose and adequate treatment. The comparative analysis of the ovarian cancers across histological profile showed 77% and 95% coincidence in histological characteristics of the neoplasm and its nature, respectively [27].

At the same time, the literature data on cytological method use in ovarian cancer diagnosis is quite controversial. Many studies traditionally consider the histological method as central in cancer diagnostics, without regarding the cytological analyses [28]. The studies limited to cytological method are characterized by the lack of information on differential diagnostics of the ovarian cancer types. At the same time, there are few works regarding only some types of ovarian cancer, such as epithelial tumors, granulosa cell tumor, etc [29]. Also, there is no single classification of ovarian cancer types, based only on cytological measures. Thus, it is extremely relevant to carry out the comparative analyses of various types of ovarian cancer and to determine the informative value of the cytological method. In this study the researchers made an attempt to analyze various cancer types (including ovarian cancer) using cytological methods of diagnostics only. Results of the present study may be integrated into similar studies on other cancer types.

Moreover, they believe that cytological research methods provide high accuracy not only in determining cancer types but also in making predictions and detecting the cancerous tumor site. The study aims to evaluate the efficiency of cytological methods in determining the exudate nature in ovarian cancer and other oncology diseases of the female reproductive system.

Materials and Methods

Materials

The research was conducted in cytology laboratories of Moscow oncologic dispensary (Russian Federation) (study group, 242 women) and oncology clinic in Sofia (Bulgaria) (comparison group, 131 women) within a period between 2012 and 2019. The total number of patients in both groups was 373 women, which were divided into two groups due to cancer type. The 1st group (study group) comprised patients with carcinomatous pleurisy. The group included patients diagnosed with breast cancer, ovarian cancer, and endometrial cancer (Table 1).

Table 1: The number of patients with different types of cancer in the study and comparison groups

Neoplasm type and its location	Study group	Comparison group
Pulmonary adenocarcinoma	0	100
Breast cancer	150	0
Ovarian cancer	86	0
Stomach cancer	0	23
Endometrial cancer	6	0
Squamous cell lung cancer	0	5
Small cell carcinoma of lung	0	3
Carcinomatosis (total amount of both groups)	242	131

The average age of patients in both groups was comparable: 38.5 ± 7.8 years vs 42.1 ± 9.0 years. Prior to the conducted research, an agreement on confidentiality and non-disclosure was concluded with each of the patients. All moral and ethical norms were followed. In the study group cancer was diagnosed while examining the material of the primary tumor with morphological methods in all cases. The 2nd group (comparison group) included women diagnosed with carcinomatous pleurisy: lung cancer and stomach cancer. All cancer cases were confirmed histologically. Furthermore, a comparative analysis of measured data and that in the dispensary register and medical histories was conducted.

Methods

Pleural fluid of cancer patients was withdrawn and provided to the cytology laboratory followed by centrifugation testing, which was performed under the standard protocol. Further the samples were stained according to the Pappenheim method.The obtained samples were studied by means of a light microscope. If standard methods did not provide a definite result, they used the Cytospin-4 cytocentrifuge model. In total, there were 35 cases of the described procedure in the study group. The researchers used the methods of immunocytochemistry with marker sets (Dako company, Danmark), which included 2 to 13 antibodies. In particular, pan-cytokeratin markers AE1/AE3, MNF116, EMA, and BerEP4 as well as monoclonal and polyclonal carcinoembryonal antigens (CEA) were used for epithelial cell analyses. Mesothelin, calretinin, and thrombomodulin were used for mesothelial cell tests. Vimentin was applied in analyses involving mesenchyme. Estrogens and progesterones were used in tests involving steroid hormone receptors. CD15 marker and cell proliferation marker Ki-67 were applied in analyses associated with the lymphocyte marker.The study included a standard staining according to the research protocol [30]. To detect the antigen-antibody reaction, they used the streptovidin-biotin system (LSAB2). Alongside with it, the polymer-based detection system (The DAKO EnVision+ System) with 3, 3'-diaminobenzidine (DAB) substrate-chromogen was used. If the reaction took place, the additional smear staining with hematoxylin was carried out.

Statistical Analysis

The calculations were performed using Statistica v. 10.0 (Stat Soft Inc.). The differences between the compared characteristics and groups were considered to be statistically significant at $p \leq 0.05$ (low level). To determine the differences, the Fisher's exact test was used. The mean and error of the mean were computed for each group.

Results

When comparing the results of histological and cytological analyses, it was found that almost all observed cases of breast cancer corresponded to infiltrating ductal carcinoma, and only a few to the lobular one (Fig. 1A).

Figure 1: Breast neoplasms (1A) and ovarian cancer (1B) differentiation

The other types of breast cancer were not found. Moreover, in 90% of cases tumor cells were identified on the serous membrane. The cytological analysis of pleural fluid revealed that tumor cells originated from the mammary gland in 67% of cases, about 12% from pleurisy, and the other cells were of adenogenous nature. Cytological analysis showed serous adenocarcinoma in all ovarian cancer cases with predominance of highly differentiated type (Fig. 1B). The other two forms were found in less than a half of cases.

Cytological analysis revealed the origin of ovarian neoplasms in 35% of cases. This is an important diagnosis criterion for initial examination. In breast cancer, some important cytological signs were specified, which could promote diagnosing the neoplasm nosology according to the cellular structures. Thus, the ductal carcinoma cases show the predominance of spherical cells (Fig. 2A), while the less common cases of breast cancer are dominated by the lobular cells arranged one after another, forming peculiar strands or chains (Fig. 2B).

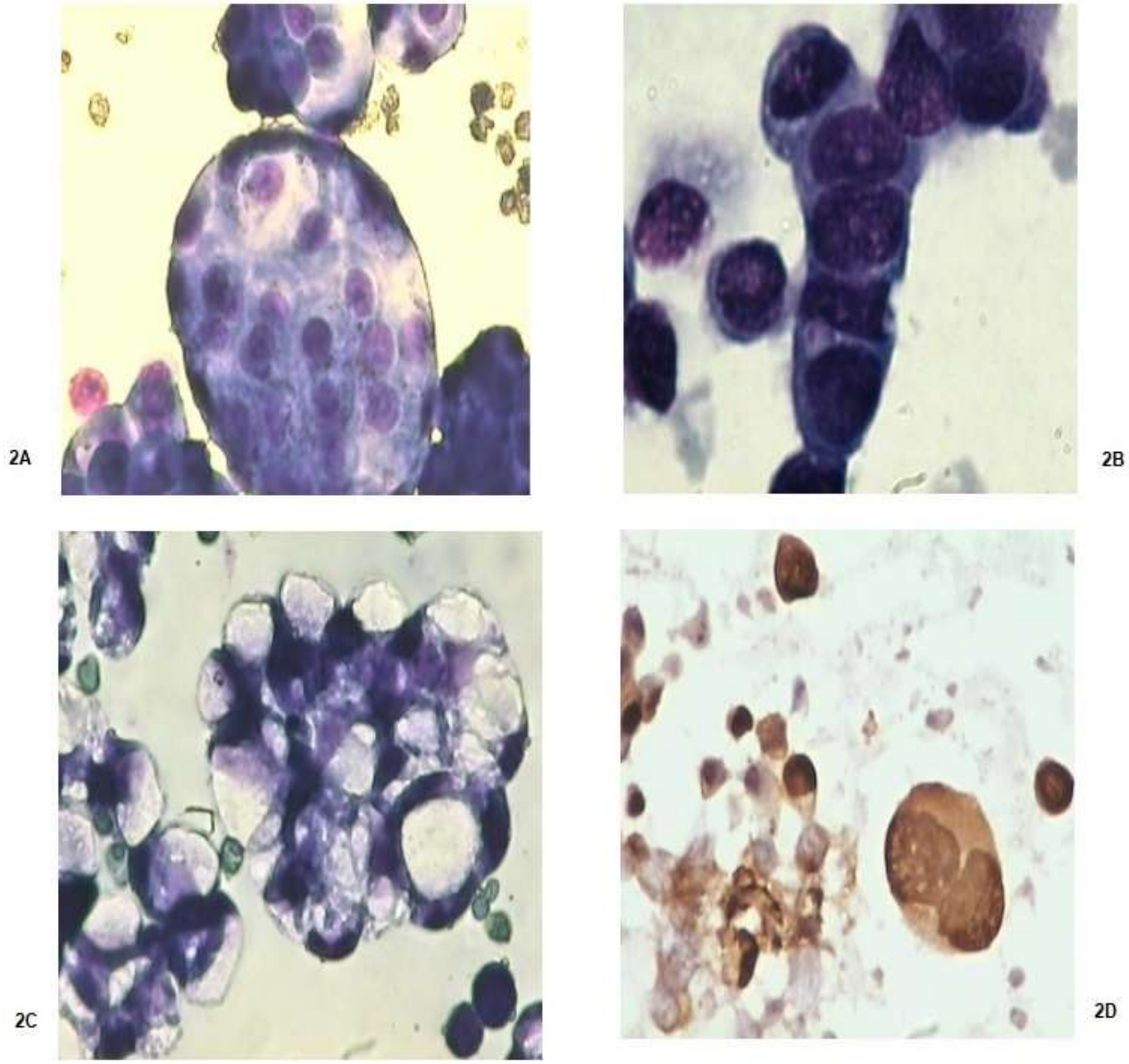

Figure.2: Cytological characteristics in breast and ovarian cancer, comparative aspect. 2A - ductal carcinoma, spherical cells in the pleural fluid (400 magnification); 2B - lobular carcinoma of the mammary gland; the differentiated lesions in the form of strands in the pleural fluid (1000 magnification); 2C - serous adenocarcinoma, one of the diagnostic cytological signs is cell vacuolization (400 magnification); 2D – signs of proliferation (Ki-67) developed after the breast cancer cell staining with hematoxylin (400 magnification). The first three images depict results of Pappenheim staining, whereas the last image displays the immunoperoxidase staining results

The visual analysis also revealed the described cells to be multinuclear and rough at the edges, which could also be an important cytological finding in the mentioned nosology. Ovarian cancer specifies another cytological aspect: excessive cell

vacuolization, more than that in carcinomatous neoplasms of any other location (Fig. 2C). This finding is important for ovarian cancer identification.

The detecting frequency of vacuolization signs is very high and reaches 65% of all cases of ovarian cancer. They consider the villiform structures at the cells' apical end, as well as sloughed clumps in cytoplasm to be less definitive cytological characteristics for the ovarian cancer diagnosing, since they were found only in 20% of cases. In endometrial cancer, the specific features similar to those in ovarian neoplasms were not detected. Cytological analysis of the other cancer types (stomach cancer in comparison group) revealed the single signet ring cells to be formed in the pleural fluid in some cases of stomach cancer (up to 20%), which was not observed in ovarian and breast cancer.

It should be noted that it is too difficult to reveal the damage site of the primary tumour in adenocarcinomas, since these neoplasms have too many similar features regardless of their site. In lung cancer, the cytological analysis did not find any specific signs. In some cases, cytological analysis focusing on the morphological cell characteristics does not allow to specify the cancer cells. Therefore, it is relevant to use a cytocentrifuge method, which enables clinicians to prepare a monolayer of cells. In particular, in 15% of small cell lung cancer diagnoses in the comparison group was achieved due to cytocentrifuge use. A centrifuge was used in 10% of ovarian cancer cases, when there was no definitive diagnosis. The immunocytochemistry methods allowed establishing a number of considerable differences between breast and ovarian cancer types (Table 2).

For instance, the use of the Ki-67 proliferation marker enabled the clinicians to obtain a significant cell response in 4 cases (in 35-45% of cases) (Fig. 2D). They established an immunopositive response of breast cancer cells to epithelial antibodies and a negative response to mesothelial antibodies (Table 2, $p \leq 0.001$). Less reliable results were obtained in cancer cell response to antibodies of the cytokeratin group in mammary gland neoplasms ($p \leq 0.05$). There was observed a significant reverse reaction between the ovarian cancer and breast cancer cells to the monoclonal antigen ($p \leq 0.001$).

The mentioned reaction enabled the researchers to distinguish ovarian and breast cancer in immunocytochemical studies, since the signs were directly opposite. The analysis of calretinin, mesothelin, and thrombomodulin they didn't show any significant differences between the cancer types of different nosologies, although negative responses were specified in 25 % of breast cancer cases and in 35% of ovarian cancer cases the response was weak.

Table 2: The differences between breast and ovarian cancer obtained by the immunocytological method

Immunoassay for tumour marker	Breast cancer		Ovarian cancer		Significance level of differences
	Number of examined patients	+ reaction, number of patients	Number of examined patients	+ reaction, number of patients	
Monoclonal CEA antibody	15	++, +++ (in 11)	38	– (in 38)	0.001
Polyclonal CEA antibody	13	++, +++ (in 13)	38	+ (in 19), ++ (in 19)	Unreliable
Vimentin	15	+ (in 6)	38	+ (in 12)	Unreliable
Epithelial membrane antigen	13	+++ (in 13)	38	+ (in 19), ++ (in 19)	0.001
Cytokeratins	15	++ (in 15)	38	++ (in 8), +++ (in 30)	0.05
Epithelial antigen Ber-EP4	15	+++ (in 15)	38	+++ (in 38)	Unreliable
Calretinin	15	- (in 15)	38	+ (in 11)	Unreliable
Mesothelin	15	- (in 15)	38	+ (in 11)	Unreliable
Thrombomodulin	15	- (in 15)	38	+ (in 11)	Unreliable
CD15 antigen (leukocyte growth factor)	15	- (in 13), + (in 2)	38	+ (in 2)	Unreliable

Notations: +++ - persistent and significant immunocytochemical response, ++ - moderate response; + - weak response; – - persistent negative response

The immunocytochemical reactions were not performed for endometrial cancer. The long-term cancer of different nosology varied in mortality rates (Fig. 3).

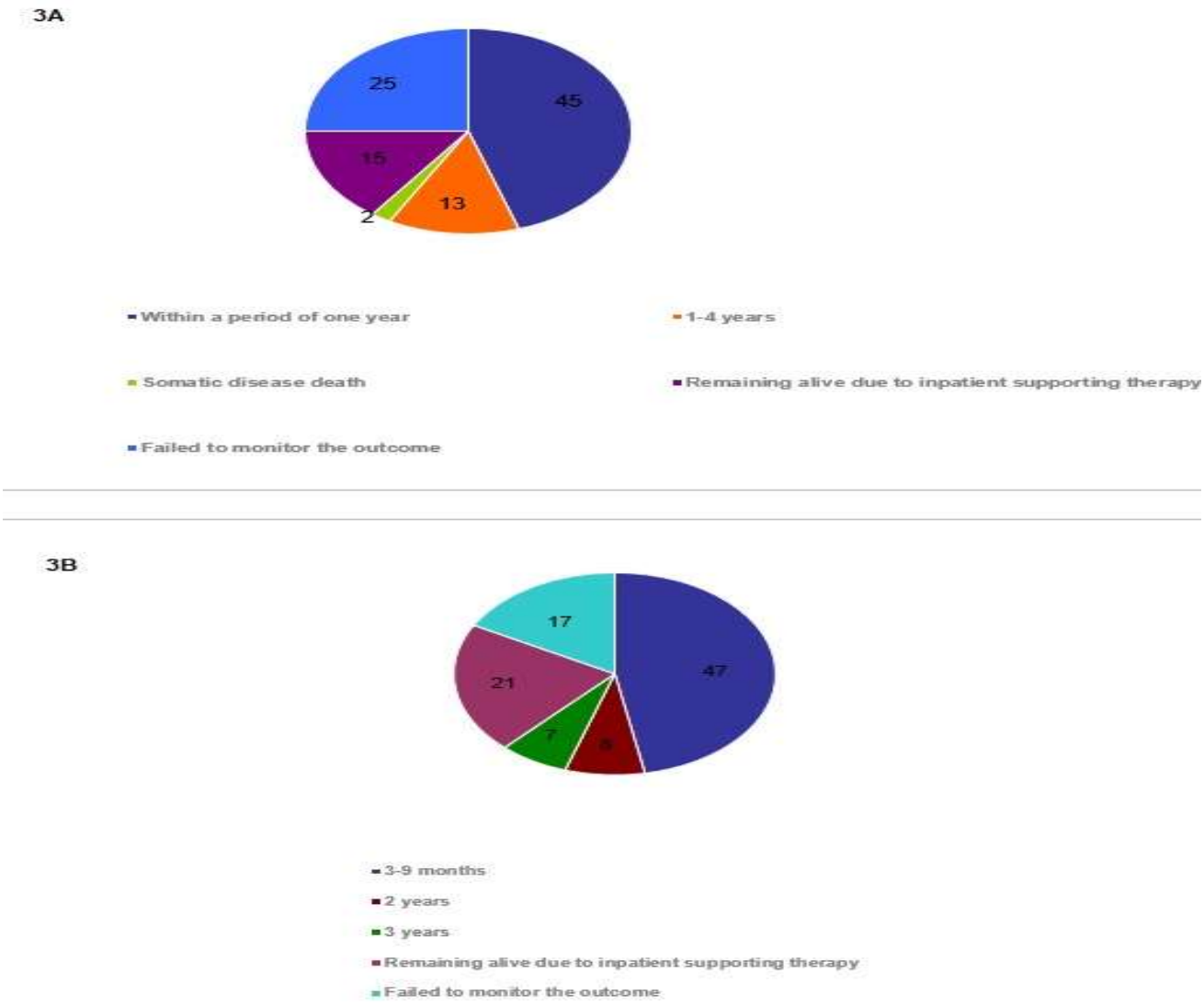

Figure 3: Breast cancer (3A) and ovarian cancer (3B) mortality rates

Over a 7-year period of patient follow-up, they have found the ovarian cancer to be a more rapid disease type (with a high mortality rate of 3-9 months) (Fig. 3B) compared to the breast cancer, where mortality occurred after a period of one year (Fig. 3A). Still, a certain number of patients survived thanks to the inpatient supporting therapy, although the difference was not significant.

Discussion

The age distribution in women with ovarian cancers is known to comply with the average population values. Nevertheless, most cases were traditionally observed in elderly and senile age [31]. In recent years, however, this cancer type showed a shift towards younger age groups [32]. The study of adequate therapy and correction methods for ovarian cancer is obviously one of the primary tasks of modern medicine. This disease affects the reproductive system, which also makes the problem socially significant. However, cancer patients are more likely to be successfully operated at early stages of disease [33]. In this regard, the advantage of the cytological method leaves no doubts due to its low invasiveness and high diagnostic accuracy not only of the cancer nosology, but also of the primary tumour site. The conducted study proved not only the above stated fact, but also showed the cytological methods to be not equally efficient in various cancer types.

The highest efficiency of the cytological methods was observed in breast and ovarian cancer in the study group. The combination of cytological methods and methods of light microscopy also provides a significant improvement in diagnostic results. Thus, they revealed signs peculiar to different cancer types: cell vacuolization (in ovarian cancer), formation of ring-shaped cells (in gastric cancer), and formation of spherical cells or cords (in breast cancer).

The efficiency level ranges from 15% to 80%, but in some cases, cytological and immunocytochemical methods were used for diagnosis specification. Some studies proved cytological methods to be more accurate than frozen section tests [34].

The diagnostic accuracy is directly related to the operation efficiency and, in some cases, with its management. In some types of ovarian tumours, such as borderline ones, less diagnostic accuracy (about 65%) can be obtained [35]. This can be explained by the fact that borderline neoplasms are characterized by implicit invasive growth.

Summing up, the use of cytological methods is important and necessary at all stages of the patient follow-up, including cases of ovarian cancer. Further research should be aimed at developing a unified classification of ovarian cancer entirely based on cytological criteria. A comprehensive therapy management, including cytological methods, should be also developed.

Conclusions

The conducted study showed that the use of cytological methods considerably advanced the diagnostics of adenogenous ovarian and breast cancer in the observation group and, in some cases, of stomach cancer in the comparison group. Thus, in 65% of ovarian cancer cases, an excessive cell vacuolization was noted. Clusters of spherical cells were observed in 80% of cases of ductal breast cancer, while in 75% of cases of lobular breast cancer there were specified cell structures in the form of strands and chains. In 20% of cases of comparison group, single signet ring cells were found, which could also be used for diagnostics.

Cytocentrifugation provides accurate diagnostics of cancer nosology and the site of the primary tumor in 20% of cases of ovarian cancer. At the same time, in 15% of lung cancer cases, this method helped to distinguish the small cell lung cancer type. The use of immunocytochemistry made it possible to detect a clear response to epithelial antibodies and a negative response to mesothelial antibodies in breast cancer ($p \leq 0.001$), while an opposite response to monoclonal antigens ($p \leq 0.001$) was obtained in ovarian cancer. Thus, cytological methods significantly enable the clinicians to specify the diagnosis of such cancer types as ovarian cancer and breast cancer.

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