Different patterns of heterogeneity in ovarian carcinoma

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Abstract

Ovarian cancer is still the leading cause of death from malignant genital tract lesions. Ovarian carcinomas represent about 90% of cancers that arise in the ovaries and are commonly diagnosed around menopausal age. This study examines different aspects of the heterogeneity of ovarian carcinomas and included 50 cases, 10 cases for each subtype. Our data showed that tumor types have distinct morphological and phenotypic patterns: high- and low-grade serous carcinoma, endometrioid, clear cell and mucinous carcinoma. The different subtypes of ovarian carcinomas have different molecular, pathological and clinical characteristics, the histological diversity of epithelial ovarian carcinoma mirroring distinct entities not just one disease.

Keywords: ovarian carcinoma, heterogeneity, morphology, prognosis.

Introduction

Ovarian cancer is still the leading cause of death from malignant genital tract lesions. Ovarian carcinomas represent about 90% of cancers that arise in the ovaries and are commonly diagnosed around menopausal age [1].

The biological mechanism of development of ovarian carcinomas, either by malignant transformation of benign precursors or “de novo” has been a subject of scientific debate for a long time. Nowadays is favored the theory of “de novo” malignant transformation of ovarian surface epithelium, and/or serous epithelium of the fallopian tube, especially the distal part by accumulation of gene alterations in at least three classes of genes: proto-oncogenes, tumor suppressor genes, and mutator genes. The emergence of independent de novo clones without a preexisting benign tumor will lead to non-cumulative mutations [2, 3]. Ovarian carcinomas are a heterogeneous group of neoplasms classified according to the histological type and degree of differentiation, each subtype characterized by genetic defects and activation of specific signaling pathways [4]. The data in the literature show that the differences in molecular, pathological and clinical characteristics could identify the subtypes of ovarian carcinomas as distinct entities [5].

In clinical practice, subjective evaluation by an experienced sonographer is most often the best criteria to appreciate an ovarian tumor. Due to the many similarities between the different types of tumors, the attention of the ultrasound examination has shifted from discerning which type of malignant tumor, to malignant versus benign, by applying a pattern recognition system. In recent years, the International Ovarian Tumor Analysis (IOTA) Group developed two types of logistic regression models. In our study, we used both the pattern recognition system and the second type of logistic regression (LR2), the latter having the advantage of being useful on all type of tumors and also offering an estimate risk for malignancy [6].

Immunohistochemical tests have a large impact on the diagnostic of ovarian carcinomas, by providing useful assessment criteria for a better reproducibility of cell type diagnosis [7], although there is still data in the literature which consider standard routine staining as sufficient for a positive diagnostic [8].

There are five major types of ovarian carcinomas: high- and low-grade serous carcinoma, endometrioid, clear cell and mucinous carcinoma [9]. Histological distinction between these different tumor types can sometimes be difficult.

This study examines different aspects of the heterogeneity of ovarian carcinomas and included 50 cases collected from the “Victor Babes” National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania, 10 cases for each subtype.

Materials and Methods

The aim of the data collection was to gather an equal number of cases from each major histological subtype (serous, endometrioid, mucinous and clear cell). We reviewed the records of the “Bucur” Maternity of “St. John” Clinical Hospital and “Victor Babes” National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, extracted data on the five types of ovarian carcinomas without including borderline tumors. We collected a total of 50 cases and grouped them...
in five groups according to their histological subtype. The pattern recognition system uses the following principle—all ovarian masses can be placed in one of five categories according to the presence of septa and a solid component as follows:

- unilocular cyst: a cyst without septa and a solid component;
- unilocular solid cyst: a unilocular cyst with a solid component;
- multilocular cyst: a cyst with at least one septum but no solid component;
- multilocular solid cyst: a multilocular cyst with a solid component;
- solid tumor: a tumor where the solid components comprise 80% or more of the tumor when assessed in two-dimensional section.

Based on the aspect, an expert assigns the tumor as benign, borderline or malignant. All 50 tumors included in the study were evaluated through this method.

The LR2 system uses the following variables: age, presence of ascites and presence of papilations with detectable blood flow, maximum diameter of largest solid component (mm), irregular cyst walls, and presence of acoustic shadows.

Thirty-two cases were prospectively assigned an LR2 score; 14 were assigned an LR2 score retrospectively and for four cases LR2 score was not possible to calculate due to lack of appropriate imagistic data. We used the IOTA Models app available for mobile devices to calculate the malignancy chance (percentage).

Patients with complex adnexal masses with blood flow showed on Power Doppler ultrasound were also evaluated with 3D ultrasound, in order to evaluate the vascularization of solid tumors and papillary projections. Using the VOCAL program, we calculated the vascularization index (VI), the flow index (FI), and the vascularization flow index (VFI) (Figure 1). A number of 23 tumors were evaluated with this method.

The immunohistochemical study included a panel of antibodies (CK7, CK20, ER, PR, CA125, CEA, CDX2, WT1, Ki67, P53, EMA, VIM, MNF116, MUC2, and MUC5) in order to provide the most accurate immunophenotype. Tumor cells stained in the appropriate cytoplasmic/membrane/nuclear position and were scored based on the fraction of positive tumor cells.

In order to corroborate the data obtained during our study we used a biomathematical method of recording data. For the biomathematical clustering analysis of patterns of immunophenotypic expression, scores were transformed into positive (red) and negative (green), depending on the threshold used. Lack of testing was marked gray. For Ki67, we used increasing shades of orange: 0–10%, 10–50%, and over 50%.

**Results**

Most of the analysis was univariate, due to the profile of our retrospective study. We stratified patients according to their age, sonographic features, histological type and immunohistochemical pattern. The median age at diagnosis was 53.77 years (range, 20 to 82 years). The variation in diameter of the ovarian tumors ranged from 8 to 17 cm, the majority exceeding 10 cm.

Out of the 50 tumors, the preoperative ultrasound using pattern recognition system had included 46 as malignant (Figure 2) and four as borderline tumors (sensitivity 92%). In the LR2 group were included 42 out of 46 of the cases diagnosed as malignant and the four cases diagnosed as borderline tumors. The LR2 performed similarly, giving a probability of malignancy greater than 50% for 41 out of 42 cases of the “malignant” (Figure 3) and for one of the “borderline” group.

In the evaluation of 3D-Power Doppler parameters, we recorded values corresponding to existing literature cut-offs for malignant tumors for the entire described index:

- VI (cut-off: 3.77): 91.3% (21 out of 23);
- FI (cutoff: 21.35): 86.95% (20 out of 23);
- VFI (cut-off: 2.103): 86.95% (20 out of 23).

Values of vascular 3D-Power Doppler parameters seem to be a promising marker in the diagnosis of complex adnexal masses.
Histology was verified by Hematoxylin–Eosin (HE) staining and we grouped all cases according to their distinct morphological and phenotypic patterns in five separate groups.

**Group 1 – High-grade serous carcinomas**

The 10 cases in this group were between 33 and 84-year-old, with a mean age of 58.3 years. The morphological profile overlapped in most cases, with complex papillary and solid patterns and the presence of psammoma bodies in 40% of the cases. Tumor cells showed markedly atypical nuclei, high mitotic index and abnormal mitotic figures. CK7 and EMA were diffusely positive in 100% of cases, while CK20 and CEA were negative in all tumors. CA125 showed a membranous pattern of staining in eight cases. Hormonal receptors positivity was low, varying between 0 and 35%. Nuclear positivity was seen for WT1 in 90% of the cases (one case was negative). P53 staining was strong and diffuse in all cases (Figures 4 and 5). Vimentin was only focally positive in two cases.

**Group 2 – Low-grade serous carcinoma**

Patients were aged 34 to 72, with a mean of 59-year-old. We identified the characteristic pattern of papillary branching and the presence of rounded cells with scant cytoplasm, mild or moderate nuclear atypia, with prominent small nucleoli. CK7, EMA and CA125 were positive in all cases. CK20, CDX2 and CEA were negative. ER and PR reaction showed a nuclear pattern of staining in all cases in more than 50% of tumor cells. P53 showed no immunoreaction, with one exception when it was focally positive, but in 30% of tumor cells. In the cluster analysis, we considered it to be negative.

**Group 3 – Endometrioid carcinoma**

The mean age of the patients in the group was smaller, 52.6, ranging from 33 to 72 years. The histological pattern was more heterogeneous. We encountered areas formed by angulated glands surrounded by desmoplastic stroma, cribriform architecture, single cell infiltration and the presence of solid areas. In two cases, there was squamous differentiation. One case showed a sex cord–stromal tumor like pattern with tubular glands and was designated as sertoliform. All cases of endometrioid carcinomas showed strong immunohistochemical reaction with CK7 and EMA, but not with CK20. Vimentin was completely negative in tumor cells in one case and showed variable staining from focal, reduced to larger areas, in all the other cases. WT1 showed focal positivity in two cases. CEA was negative (Figures 6 and 7).

**Group 4 – Mucinous carcinoma**

The mucinous carcinoma showed the smallest median age of diagnosis, 52 years and also the broadest age interval (range, 20 to 80 years). The histological pattern showed haphazard infiltrative well-differentiated glands with marked cytological atypia and necrotic debris in glandular lumens. One case showed areas of borderline mucinous tumor adjacent to areas of carcinoma. The tumors were characterized by diffuse expression of CK7 in all cases and variable expression of CK20 – four cases were negative, one showed focal reduced positivity and the rest were positive. When comparing patterns of immunopositivity, the CK7 reaction showed a more intense reaction. The hormone receptors were negative in most cases. CEA was positive in five cases (Figures 8 and 9). MUC2 was negative in most cases, while MUC5AC showed diffuse or focal positivity in eight cases.

**Group 5 – Clear cell carcinoma**

The patients with clear cell carcinoma had a median age similar to the previous one (52.7 years), but the range was narrower (35 to 72 years). The histological pattern was heterogeneous (papillary, tubulocystic, and solid) with polyhedral tumor cells with clear cytoplasm and hobnail cells with nuclear atypia ranging from minimal to marked and rare mitoses. Four cases showed a particular pattern, one with an adenofibromatous component and three with unusual morphologic characteristics, similar to serous and endometrioid carcinoma. These four cases all showed positivity for hormone receptors in a higher percentage. The clear cell carcinoma showed diffuse positivity with CK7, EMA and MNF116 and were negative for CA125, ER and WT1. Ki67 showed values around 30%, with only three cases over 50% and one under 10% (Figure 10).

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**Figure 2 – Serous cystadenocarcinoma, 2D (A) and 3D (B) images. LR2 score showed a 92.1% probability of malignancy.**
Figure 3 – Serous cystadenocarcinoma: 2D image. LR2 score showed an 86.1% probability of malignancy.

Figure 4 – Serous ovarian carcinoma – positive reaction for cytokeratin 7, 100×.

Figure 5 – Serous ovarian carcinoma – positive reaction for p53, 40×.

Figure 6 – Endometrioid ovarian carcinoma – positive reaction in the stroma and few tumoral cells, 200×.

Figure 7 – Endometrioid ovarian carcinoma – positive reaction for estrogen receptors, 200×.

Figure 8 – Mucinous ovarian carcinoma. HE staining, 200×.
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Discussion

The ovary can develop a series of neoplastic transformation, with different morphological features, some of which identifiable in standard examination, but in order to correctly diagnose ovarian tumors, an integrated diagnostic approach combining clinical, radiological and histological findings is needed. The different subtypes of ovarian carcinomas have different molecular, pathological and clinical characteristics, their histological diversity mirroring thus distinct entities not just one disease [10].

Despite the rise in knowledge of the pathways involved in ovarian carcinogenesis, which shows the considerable heterogeneity of this neoplasia, most therapies for epithelial ovarian cancer are uniform. In order to assess the heterogeneity of cases included in the study, we created five sets of records, taking into consideration the epidemiological, clinical, morphological, phenotypical and prognostic characteristics.

Heterogeneity of age of diagnosis

When stratifying into younger and older age groups (cut-off 40 years), the majority of patients in our study were over 40-year-old, ovarian carcinoma being described as a postmenopausal lesion. The cases of ovarian carcinoma in young people were mostly mucinous, well-differentiated type, which is consistent with the data from the literature [11]. Tung et al. showed that the influence of ovulation cycles on mucinous tumors is smaller than in non-mucinous tumors [12].

The onset of ovarian carcinoma before 25-year-old is very rare and it has implications on therapy due to the necessity of fertility conservation. In our study, we encountered two cases of ovarian carcinoma under 25 years and they were both mucinous subtype.

Heterogeneity of ultrasound aspects

The sonographic characteristics described were similar to the data in the literature. Large size ovarian tumors are more likely associated with malignancy [13]. Serous cystadenocarcinoma usually have a multilocular aspect sometimes with thick septations and papillary projections. Endometrioid carcinomas are cystic structures with papillary projections but may also appear as solid structures. Mucinous carcinomas present as multiloculate cysts with hypoechoic content and papillary excrescences [14]. Clear cell carcinomas have non-specific ultrasound aspects, but more frequent are cystic structures.

Heterogeneity of histological features

The different growth patterns and cell types that may be encountered, which form the basis of the histological subtypes, correlate with the molecular abnormalities that form the basis of therapeutic response in order to provide reproducible, superior prognostic information [15].

The correct identification of histopathological features has been recently emphasized by Reyes et al., who found sensitive and specific characteristic morphological features of high-grade serous carcinomas of the uterine adnexa correlated with BRCA1 deficiency [16].

The most common subtype of ovarian cancer is serous (50–60%), followed by endometrioid (25%), clear cell (4%), and mucinous (4%) carcinoma [17].

Serous carcinoma is the most common form of malignant ovarian neoplasm (~50%), consisting of epithelial cells similar to those in the fallopian tube. The remaining subtypes are encountered in smaller proportions (~10%). Mucinous ovarian carcinomas show histological cysts lined by mucin-secreting epithelial cells that may resemble either endocervical or intestinal epithelium [18].

There are two distinct types of ovarian serous carcinoma, low-grade and high-grade, with different pathogenesis, usually diagnosed in advanced stages. The low-grade is rare, arises from a serous borderline tumor and is usually associated with b-raf and k-ras mutations. High-grade serous carcinomas are usually associated with TP53 mutation and they believed to develop from the epithelium of the distal fallopian tube [19].

Low-grade serous carcinomas are characterized by indolent growth and resistance to chemotherapy. Generally, the recurrences maintain the well-differentiated histological appearance of the primary tumor, and very rarely progress to high-grade serous carcinoma [20].

Malignant mucinous ovarian tumors show a heterogeneous pattern, different types of benign, borderline and carcinomatous areas coexisting in the same lesion.

Another example of heterogeneity in ovarian carcinomas is the broad spectrum of histological grade present...
sometimes in the ovary. Zheng et al. showed that the morphologically less aggressive lesions accompanying ovarian carcinoma have molecular abnormalities for at least a predisposition to a high-grade phenotype [21].

Heterogeneity of phenotype

Ovarian carcinomas are genotypically and phenotypically heterogeneous diseases, with variable clinical courses. Different clinical and histological phenotypes of ovarian carcinomas are driven by specific genes. Biochemical pathways driven by these genes should distinguish between different types of ovarian carcinoma. Gene expression profiling was used to characterize ovarian cancers, to provide a distinct clinicopathologic characterization of these tumors.

The distinctive immunohistochemical features of each subtype of ovarian carcinoma are a valuable help in differentiating lesion inside the group but also diagnosing metastatic lesions. The ovary is a common site of metastases, with secondary carcinomas representing approximately 10–30% of all ovarian malignancies [22].

The expression of CK7 was present in all cases in our study group, but it can be absent in some mucinous carcinomas [23]. In these cases the differential diagnosis with a metastasis, especially colorectal is compulsory. The expression of CK20 shows a more heterogeneous pattern. While negative in most of the cases, the mucinous subtype shows positivity for CK20, with different intensity, but always lowers than the CK7 positivity. Together with CEA and CDX2, CK20 is very useful in differentiating metastatic lesion. Primary ovarian carcinomas, with the exception of mucinous subtype, are CK7+/CK20-/CEA- and CDX2-negative [24].

Another useful biomarker in the differential diagnosis is CA125. Cancer antigen (CA) 125 was positive in over 50% of cases. When comparing degrees of intensity of the immunoreaction, we noticed an intense staining of serous tumoral cells comparing with mucinous immunoreactivity. CA125 shows strong and diffuse staining of serous and endometrioid ovarian carcinomas, while in mucinous tumors the positivity ranges from 0–50% [22].

None of these biomarkers has individual value if not interpreted together with the rest of the panel, histological features and clinical data [25].

Similar to the results of our study, WT1-positive tumors are described to be more often high-stage, high-grade and have a higher proliferation index (higher Ki67 positivity) [26]. When comparing to low-grade serous carcinoma, high-grade had a higher expression of Ki67 and p53 and steroid receptor expression was present in both types of carcinomas.

When comparing the expression of p53, we found different patterns of staining which correlated with the histological subtype. The high-grade aggressive tumors showed high intensity of the immunostaining. Although the correlation between grade, stage and p53 was described in the literature, there are still discrepancies [27].

Focal, weak, heterogeneous pattern of immunostaining for p53 is not an indicator of mutation (‘wild-type’ p53) and is more frequently present in low-grade serous carcinoma.

We created a virtual pattern of analysis (micro-grids) of proteins encoded by genes involved in tumor initiation and progression. Following the introduction of data, the generated matrix showed each reference parameter corresponding to a selected color. Depending on the selected color range, the effective parameters can be identified visually and the clustering of different subtypes of tumors identified (Figure 11).

Heterogeneity of prognostic

The data from the literature describe differences in prognostic and therapeutic response among histology types. Serous and endometrioid carcinomas are very chemoresponsive, while mucinous and clear cell tumors are considerably more resistant to standard therapy [17].

Low-grade serous carcinomas do not respond well to traditional chemotherapeutic agents in contrast with high-grade serous carcinoma, which usually responds well initially to chemotherapy but which commonly recurs. Although the prognosis of mucinous carcinoma is excellent, even in the presence of intraepithelial carcinoma or microinvasion, advanced ovarian mucinous carcinomas have poorer prognosis than the serous type [28, 29].

Advanced stage (III/IV) correlated with histological criteria of clear cell or invasive mucinous carcinomas are indicators of poor prognosis [30].

Conclusions

The present study shows the heterogeneous pattern of development and phenotypic profile of ovarian carcinomas. Prevention of new ovarian carcinomas depends on understanding the natural history of these diseases. Modern methods of investigation of ovarian carcinogenesis (clinical, pathological and molecular) represent the basis for prevention, early detection and treatment of these tumors.

Conflict of interests

The authors declare that they have no conflict of interests.

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Figure 11 – Pattern analysis dendrogram. CCC: Clear cell carcinoma; MC: Mucinous carcinoma; EC: Endometrioid carcinoma; LGSC: Low-grade serous carcinoma; HGSC: High-grade serous carcinoma.
References


