QUANTIFICATION OF THE PROLIFERATIVE ACTIVITY IN THE EPITHELIAL TUMOURS OF THE OVARY

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Abstract: The diagnosis and the treatment of the ovarian tumors grading don’t present always significant correlations with the surviving chance. From this reason, the authors have proposed to study the proliferative activity in the main types of primitive epithelial tumors of the ovary (benign, borderline and malign) by computerized quantitative methods and to establish the significance of the quantified parameters in establishing the degree of tumor risk. It was used an interactive video digital quantitative program estimating the proliferative activity by stereological methods. The results were processed statistically and it was appreciated the degree of correlation with the tumor evolution. In the studied types of ovarian epithelial tumors, the proliferative activity represents an expressive indicator in establishing the malignancy degree. The comparative study of all types of EOT was allowed to establish some reference values for assessment of risk degree. The increase of mitotic activity index over 8 mitosis / 10 hpf represents a criterion of high-risk in borderline EOT, and over 30 mitosis / 10 hpf attests doubtlessly the malignancy. The index of the mitotic activity is a method which can be used in the routine examination of the epithelial tumors of the ovary, firstly because is significant, but and rapid and easy to apply.

INTRODUCTION

The diagnosis and treatment of the epithelial ovarian tumours (EOT) face difficulties because the FIGO stages can be used only in the initial clinical diagnosis which is based on subjective criteria. Moreover, the histological grading doesn’t present always significant correlations with the surviving chance (1). In the situation in which are used only the methods of clinical grading, 8 - 20% of female patients in the first stage have more extensive than those diagnosed, and the death rate after 5 years from the surgery is 35 % (2).

In the situation in which the clinical staging and qualitative microscopic analysis can’t make the lesion prognosis, it has been used more and more the quantitative microscopic aspects establishing objective norms which ensure a satisfying diagnosis and an adequate therapeutical decision. In the early ’90, Back (3, 4) has shown that morphometry can be used for the differentiation of the serous and mucinous borderline tumours from the malignant ovarian tumours.

The majority of the quantitative researches have followed especially the dimensions of the tumoral cells, focusing on the nucleus of the cell (5, 6, 7). The most important factor of the prognosis seems to be the percentual volume of the epithelium, as it result from ours stereological researches (8). The morphometry of the nucleus has a less predictive value, because only some parameters are significant (the diameter, the short axis). Our previous studies (9) support the value of the nuclear morphometry, but also it can use the nucleus perimeter, because appreciate indirectly the nucleus shape which has a certain predictive value.

In this issue, the authors have proposed to quantify the proliferative activity in the main types of the EOT through computerized quantitative methods and to establish its significance in assessment of the degree of tumour risk.

MATERIALS AND METHODS

The study had contain initially 216 cases of ovarian lesions, from which 90 cases of ovarian tumours which the origin in surface epithelium and stroma was selected and obtained between 2001-2005 in the Ist Clinic of Obstetrics and Gynecology of Targu Mures and the IIIrd Clinic of Obstetrics and Gynecology of Iasi. The age of the patients was between 19 and 77 years.

The quantitative study was made on sections obtained from ovarian tumours which the origin in the surface epithelium, separatedly for each histological type (serous, mucinous, endometrioid, with clear cells, undifferentiated and with transitional cells), being eliminated from the beginning the ovarian cysts, benign and malignant ovarian tumours with the origin in the sexual cords, germinal cells.

In each case, on reprezentative sections was estimate the changes in the constitutive structures and the interrelations between them. The quantitative measurements was made by a single person in order to avoid the effects of variations among many observers. The images were taken over the microscope with an acquisition system and a proffesional programme PRODIT 5.2. was applied. This digital interactive programme allows the performance of a numerous quantifications by choosing from the menu the desired quantitative method.

The assessment of the proliferative activity was made on 50 consecutive fields using an 10x ocular, a 40x objective with 0.75 numerical aperture and a circular field with 450 μm in diameter and an area of 0.159043 mm^2. The mitotic activity index (MAI) was quantified and represents the total number of mitotic figures on 10 hpf (high-power field). The results are calculated automatically by the software for each tumoral type.

RESULTS AND DISSCUSIONS

In the first stage of the issue we have examined qualitatively the slides for histopathologic diagnosis analysing the EOT on type: serous, mucinous, endometrioid, with clear cells, with transitional cells and undifferentiated (fig. 1).

From the 90 examined cases, 89 % were bilateral and only 11 % were unilateral. The majority of the bilateral tumours was represented by serous type, in concordance with speciality literature. Tavassoli (10) mentions the fact that two thirds of serous adenocarcinoma, a half of serous borderline tumours and 80 % of benign serous tumours are bilateral. From the other categories of EOT, only the tumours with transitional cells are bilateral in 28 % of the cases, the rest in only 5 % of cases. The unilateral or bilateral appearance of the tumour is an important predictive factor and is comprised in the stadialization of the malignant and borderline tumours.

The majority of the tumours has a malignant character (in 52 of cases) and the number of borderline tumours the lowest (only 14 cases). In concordance with the data from speciality literature, most of the examined tumours were serous type (43 %), followed by the mucinous type (29 %) and endometrioid (14 %), the other microscopical types being found more rarely. In the study performed by Prat (11), the serous tumours were the most frequent, representing 30 – 40 % of EOT, followed by the mucinous type which represent 10 - 15 % and endometrioid type 2 -4 %. In the studied case-book record we haven't found any case of squamous cell tumour or epithelial mixed tumour.

A prognostic importance in the case of serous borderline tumours with peritoneal implants has the separation of them in invasive and non-invasive. 35 % of the borderline serous tumours weren't associated with the presence of the implants, and from 65 % of cases with peritoneal implants, in 25 % of cases the implants were non-invasive epithelial type and in 5 % of cases were non-invasive desmoplastic, and in 35 % of cases the implant were invasive. The non-invasive implants, no matter the type, don't modify the prognosis of the borderline serous tumours, but the implants of invasive type (microinvasion) worsen the prognosis (12).

The degree of histological malignancy represents also an important factor of prognosis, the majority of the studied tumours (50 %) have the third degree of malignancy and a reserved prognosis. In the borderline EOT the nuclear atypia is often present in different degree and a variable number of mitosis, sometimes very numerous (the mucinous borderline tumours of intestinal type). In the malignant tumours, the appearance of the tumoral nucleus is characteristic for each tumoral type and there are no doubts regarding the affiliation to the ovarian cancers group.

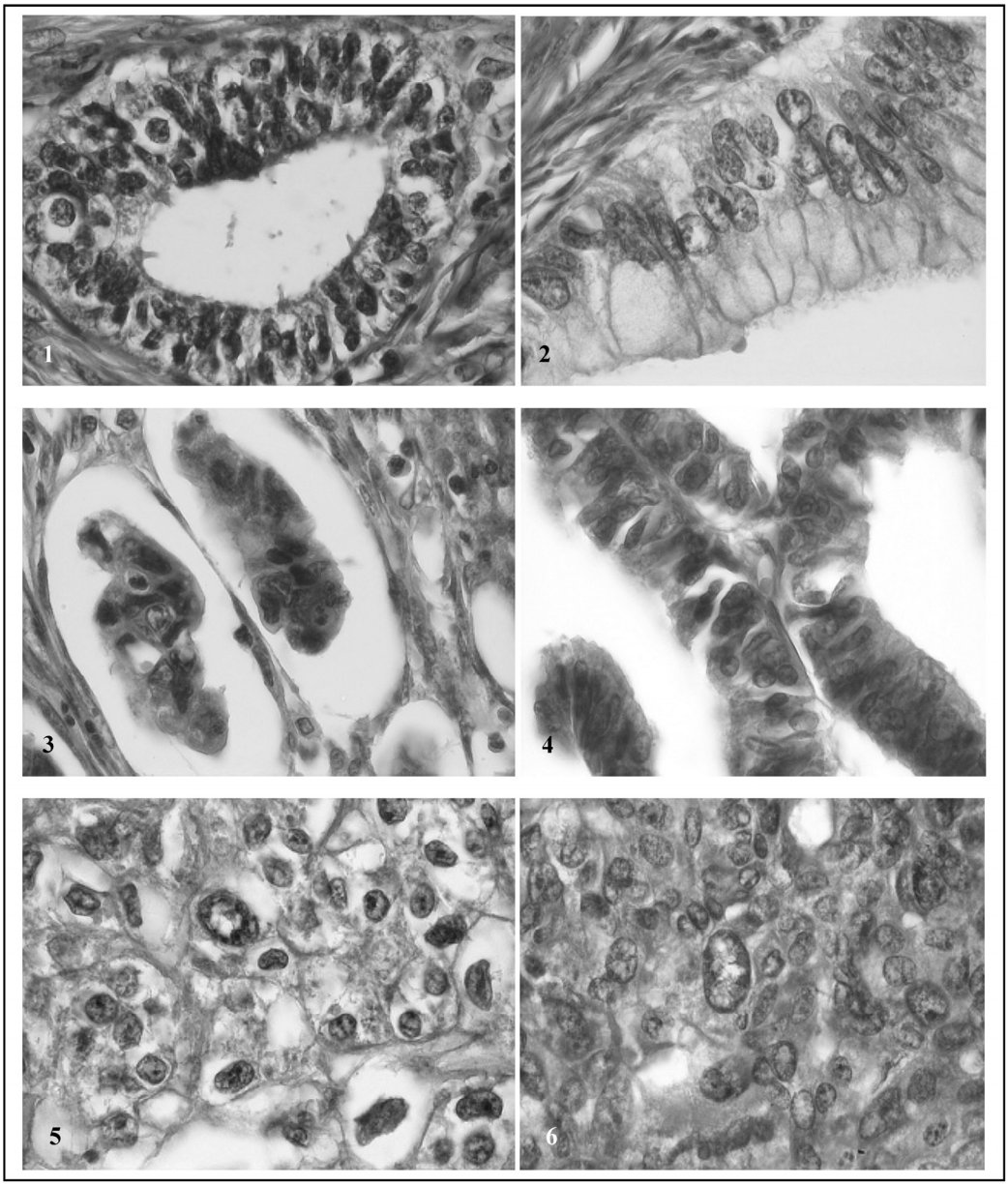

Fig. 1. Aspects of the examinated tumours: 1. endometrioid adenofibroma, 2. borderline mucinous tumour, intestinal type, 3. borderline mucinous tumour, intestinal type with microinvasion, 4. endometrioid adenocarcinoma, 5. adenocarcinoma with clear cells, 6. serous adenocarcinoma. H&E stain, x400.

In the second stage, for the each tumoral type we have selected the representative slides for the quantitative study and we will present concisely the quantified values for each type and subtype (fig. 2).

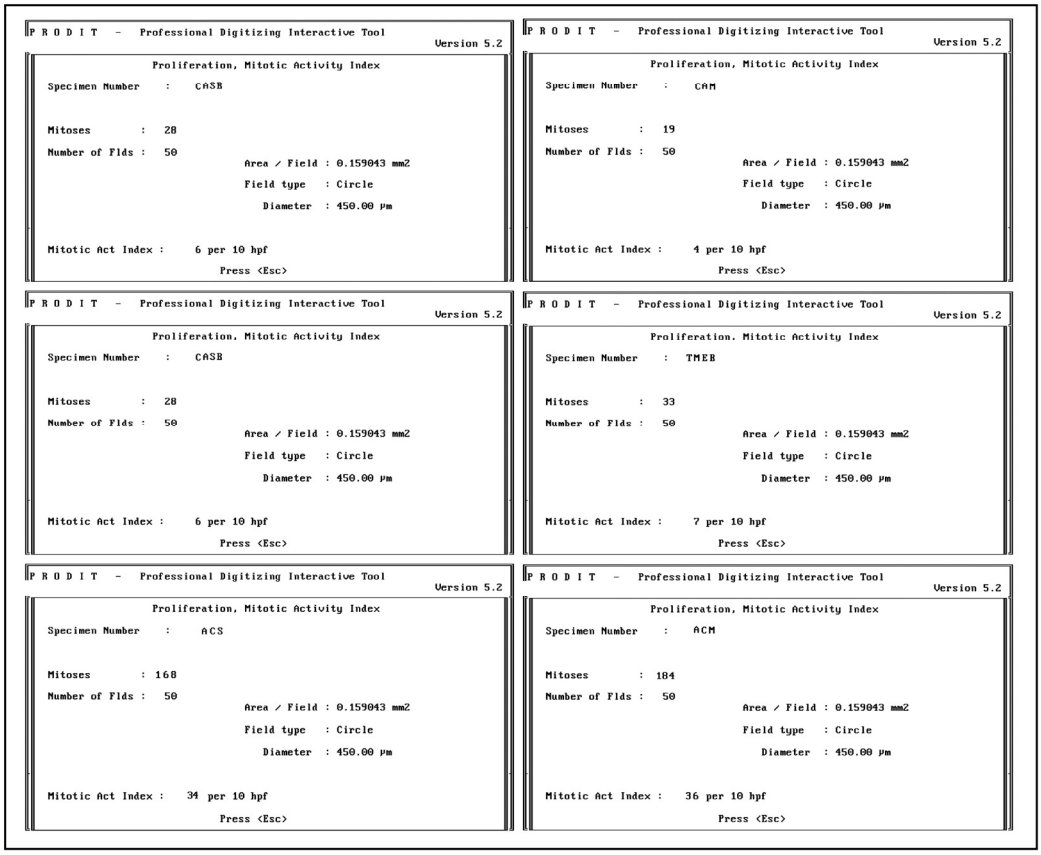

Fig. 2. The illustration of the quantification reports of the proliferative activity in benign (SCA and MCA), borderline (SBCA şi MEBT) and malignant (SAC şi MAC).

In the **serous EOT**, MAI presents the following medium values for each histopathological type:

a. benign tumours:

- serous cystadenoma (SCA) – 2 mitosis / 10 hpf,
- surface serous papiloma (SSP) – 3 mitosis / 10 hpf,
- serous adenofibroma (SAF) – 4 mitosis / 10 hpf,

b. bordeline tumours:

- serous borderline cystadenoma (SBCA) – 6 mitosis / 10 hpf,
- surface serous borderline papiloma (SSBP) – 4 mitosis / 10 hpf,
- serous borderline cystadenofibroma (SBCAF) – 17 mitosis / 10 hpf,

c. malignant tumours:

- serous adenocarcinoma (SAC) – 34 mitosis / 10 hpf.

The medium values of MAI emphasize significant changes in serous tumors, low values in the benign serous tumours and a little rise in the borderline forms (SBCA and SSBP), especially marked in the SBCAF, but the most significant rise is in the SAC.

In the **mucinous EOT**, MAI presents the following medium values for the each histopathologic type:

a. benign tumours:

- mucinous cystadenoma (MCA) – 4 mitosis / 10 hpf,

b. borderline tumours

- mucinous endocervical borderline tumour (TMEB) – 7 mitosis / 10 hpf,
- mucinous endocervical borderline tumour with microinvasion (MEBTM) – 17 mitosis / 10 hpf,
- mucinous intestinal borderline tumour (MIBT) – 6 mitosis / 10 hpf,
- mucinous intestinal borderline tumour with microinvasion (MIBTM) – 18 mitosis / 10 hpf,

c. malignant tumours

- mucinous adenocarcinoma (MAC) – 36 mitosis / 10 hpf.

The MAI presents significant changes in the mucinous tumours. It has very low values in mucinous cystadenoma, rises slowly in the borderline forms without invasion (MEBT, MIBT), is especially marked in mucinous borderline tumours with microinvasion (MEBTM and MIBTM), but the most significant rise is in the MAC.

In **endometrioid EOT,** the medium values of the MAI for each histopathologic type are:

a. benign tumours:

- endometrioid adenofibroma (EAF) – 6 mitosis / 10 hpf ,

b. malignant tumours:

- endometrioid adenocarcinoma (EAC) – 36 mitosis / 10 hpf,
- carcinosarcoma or malignant mullerian mixed tumour (CS) – 34 mitosis / 10 hpf,
- endometrioid stromal sarcoma with low-degree (ESS) – 25 mitosis / 10 hpf.

The significant changes of the MAI in the endometrioid EOT emphasize very low values in EAF, increases obviously in the malignant forms, the most evident is in EAC and the less marked is in ESS.

In **EOT with clear cells**, MAI presents the following medium values for each histopathologic type:

a. benign tumours:

- cystadenofibroma with clear cells (CAFCC) – 4 mitosis / 10 hpf,

b. tumori bordeline:

- borderline cystadenofibroma with clear cells (BCAFCC) – 13 mitosis / 10 hpf,

c. malignant tumours:

- adenocarcinoma with clear cells (ACCC) – 39 mitosis / 10 hpf.

The MAI presents significantly different values in EOT with clear cells: very low values in CAFCC, increases obviously in the borderline forms and is the most evident in ACCC.

In **EOT with transitional cells**, MAI presents the following medium values:

a. benign tumours:

- benign Brenner tumour (BBT) – 3 mitosis / 10 hpf,

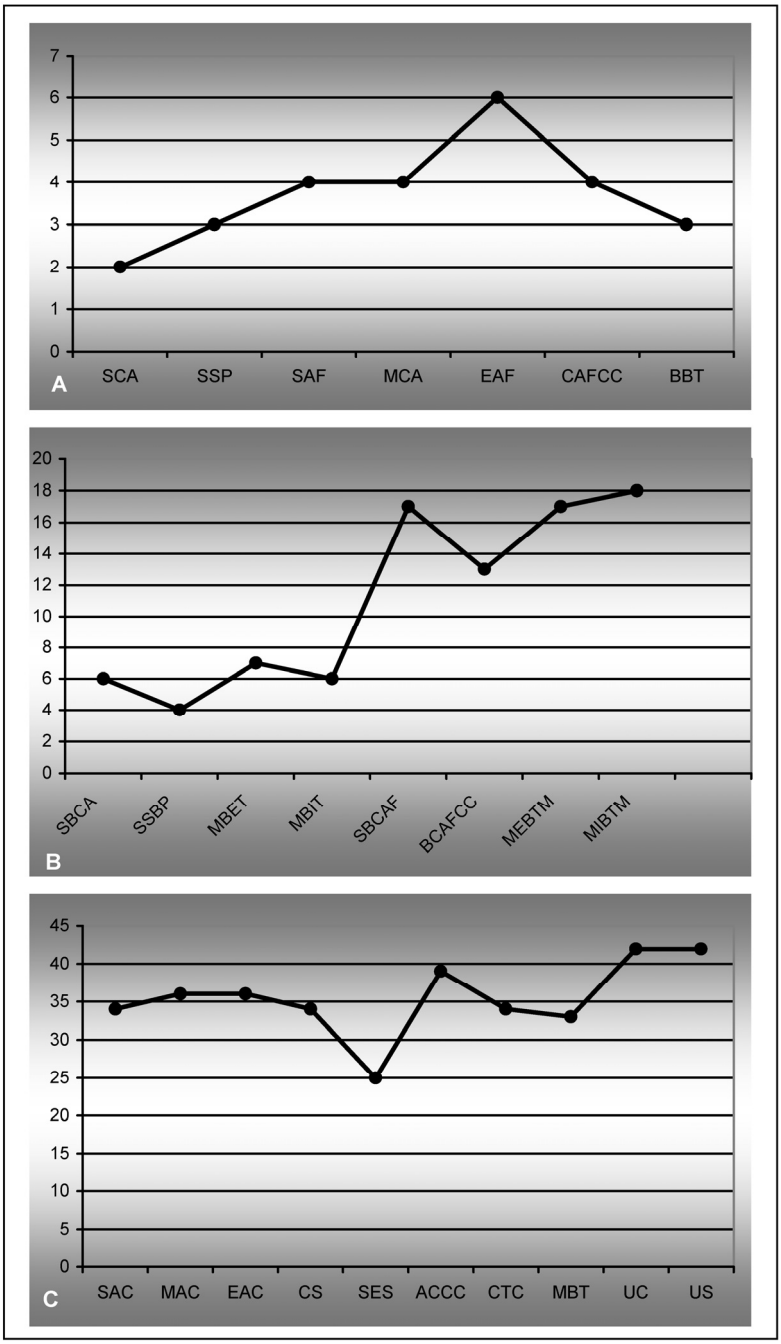

Fig. 3. The graphical reprezentation of MAI in benign (A), borderline (B) and malignant (C) EOT.

b. malignant tumours:

- carcinoma with transitional cells (CTC) – 34 mitosis / 10 hpf,
- malignant Brenner tumour (MBT) – 33 mitosis / 10 hpf.

In EOT with transitional cells, MAI varies apparently with very low values in BBT and very high values in the malignant forms.

In the **undifferentiated malignant tumours,** the increase of MAI is the most marked from all the types of EOT:

- undifferentiated carcinoma (UC) – 42 mitosis / 10 hpf,
- undifferentiated sarcoma (US) – 39 mitosis / 10 hpf.

The graphical reprezentation of MAI in benign EOT (fig. 3A) had praised generally values between 2 and 4 mitosis / 10 hpf, with an exception, EAF which has the highest proliferative activity among the other benign EOT (6 mitosis / 10 hpf), so a higher risk.

The analysis of the graphical reprezentation of MAI in borderline EOT (fig. 3B) had shown generally values between 4 and 8 mitosis / 10 hpf, with an exception, SBCAF which presents the highest proliferative activity among the other borderline EOT (17 mitosis / 10 hpf), analogous with the endometrioid forms with microinvasion, so with a high risk of malignancy. In the borderline EOT the assessment of risk degree is more difficult qualitatively, often only quantifications can offer objective data.

The graphical reprezentation of MAI in malignant EOT (fig. 3C) had praised generally values upon 30 mitosis / 10 hpf, with the exception of stromal endometrioid sarcoma low-degree which has the lowest proliferative activity among all the malignant EOT (25 mitosis / 10 hpf). The most intense proliferative activity is found in the undifferentiated types, in decreasing order the undifferentiated carcinoma and after undifferentiated sarcoma.

The quantification of the proliferative activity had shown that it can be a crucial criteria for the precocious assessment of the evolution. At the same time with the increase of the risk in the borderline tumours, especially in those with microinvasion, the MAI increase significantly and has the highest value in the undifferentiated types (42 mitosis / 10 hpf).

The comparative study of all types of EOT was allowed to establish some reference values for assessment of risk degree. The increase of MAI over 8 mitosis / 10 hpf represents a criterion of high-risk in borderline EOT, and over 30 mitosis / 10 hpf attests doubtlessly the malignancy.

In the serous borderline tumours, the peritoneal implants of invasive type (microinvasion) worsen the prognosis, this fact being sustained by the increase of the MAI values in SBCAF (17 mitosis / 10 hpf).

Dietel şi Hauptmann (13), during the performance of the morphometry of the serous EOT recomend the best morphometrical indicators which confer malignancy conviction: the mitotic activity index over 30, the percentual volume of the epithelium over 60 % and short nuclear axis. The same authors (13) consider that only the borderline EOT with only 4 mitosis / 10 hpf can have a very favourable prognosis. In our case book record the limits are larger, between 4 and 8 mitosis / 10 hpf, the microinvasion being associated with higher values (10 mitosis / 10 hpf).

Correlating the personal study with the existing data from the literature, we encourage the necessity of the evaluation of the proliferative activity before begenning of the treatment, not only for the more beneficial effects for the patients, but also for the assessment of the last ones (14).

The quantification of the proliferative activity in EOT had shown that it can be used as an additional technique in the differentiation of borderline from malignant tumours, being a crucial criteria for the precocious assessment of the evolution.

CONCLUSIONS

The comparative study of all types of EOT was allowed to establish some reference values for assessment of risk degree. The increase of MAI over 8 mitosis / 10 hpf represents a criterion of high-risk in borderline EOT, and over 30 mitosis / 10 hpf attests doubtlessly the malignancy.

The mitotic activity index (MAI) is a method which can be used in the routine examination of the EOT, firstly because is objective, but and rapid and easy to apply.

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