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DNA PLOIDY AS AN INDICATOR OF DIAGNOSTIC ACCURACY IMPROVEMENT AT EARLY STAGES OF EPITHELIAL OVARIAN CANCER

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Abstract

This study discusses the potential role of DNA ploidy measurement in the ThinPrep smears as an indicator of improvement of diagnostic accuracy in the investigation of patients with borderline and malignant common epithelial tumours of the ovary in stages Ia – IIc. Our study was carried out in 28 patients with borderline malignant and malignant epithelial tumours of the ovary in Ia to IIc stage. For ThinPrep cytology two smears (1 stained by Papanikolaou and 1 by Feulgen) were prepared. In the Feulgen stained smears DNA ploidy measurements were performed using SAMBA 2004 Image Analyzer System (Alkatel™, Grenoble, France) according to the standard protocol. Seven cases (25%) of our material were aneuploid and the remaining 21 cases (75%) were euploid. From 28 cases of our material 12 cases were borderline malignancies. All our patients with diploid status of DNA (n=21) had a good response to therapy. From results of our investigation we can report that diploid tumours had better prognostic value compared with aneuploid tumours.

DNA ploidy can be measured in ThinPrep cytological smears and can help to improve the diagnostic accuracy of cytological examination, especially in cases of early stages of epithelial ovarian tumours. Improving the diagnostic accuracy in early stages could help to choose the correct management for these patients.

***Keywords*:** epithelial ovarian cancer, DNA ploidy, ThinPrep (TP), liquid based cytology.

Introduction

Ovarian cancer is the fourth most frequent cause of cancer-related death in women and account for 5% of all cancer deaths. Epithelial cancer is the most common ovarian malignancy and, as they are usually asymptomatic until metastasized, patients present with advanced disease in more then two-thirds of cases [*Ozols R. et al*., 2000].

The outcome of patients with epithelial ovarian cancer can be evaluated in the context of prognostic factors, which can be grouped into stage of disease, histological types and grade, volume and residual disease, biologic, clinical factors, etc.

The 5-year survival of patients with epithelial ovarian cancer is directly correlated with the tumour stage. In early studies the reported 5–year survival in patients with stage I disease was approximately 60 %- 80 %. However, current studies utilizing a comprehensive staging laparotomy demonstrate that some subsets of patients with stage I disease have a 90 % 5-year survival. Similarly, initial studies in patients with stage II disease reported the range of 5-year survival

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about 40%-50%. Stage II disease frequently is upstaged to stage III disease, particularly when patients present with large-volume disease in the pelvis [*Young R. et al., 1990*].

Several biologic factors have been correlated with prognosis in epithelial ovarian cancer. A hypothesis suggested that ovarian cancer is a polyclonal disease arising at multiple sites that shared a susceptibility to events leading to malignant transformation. Multivariate analyses have demonstrated that ploidy is an independent prognostic variable and one of the most significant predictors of survival. However, the role of ploidy analysis for predicting the behaviour of epithelial ovarian tumours remains controversial.

Using flow and image cytometry allows to detect a DNA ploidy. Flow and image cytometric analyses also provide data on the cell cycle, and the proliferation fraction (S-phase) determined by this technique has correlated with prognosis in some studies. A study of W. Gajewski and co-authors discussed the prognostic value of DNA ploidy analysis in early-stage disease, and whether this technique might help to identify those patients at significantly higher risk of recurrence, and who might benefit from adjuvant therapy, is a subject highly debated. At 10-year follow-up, the survival was 100 % for nine patients with diploid tumours [*Gajewski W. et al., 1994*].

Differential diagnostics of borderline ovarian tumours and ovarian carcinomas is generally based on morphological criteria, which are not always sufficient for final diagnosis. However, it is important to separate these from their invasive counterparts because of their superior prognosis [*Russack V., 1994*]. The purpose of this study is to investigate whether image cytometric analysis of cellular DNA content acts as a useful adjunct to the cytological and histopathological diagnosis of borderline and malignant epithelial ovarian tumours.

During the last years a new cytological method was presented for use in clinical practice.

The ThinPrep Processor (Cytyc Co., Marlborough, MA) is a thin-layer preparation device that has been gaining in popularity in the recent years. It is used to prepare slides from cell suspensions collected in a preservative liquid (Cytolyt® solution).

In gynaecological and non-gynaecological specimens, the ThinPrep® method appeared to improve the diagnostic accuracy and the quality of the smear, reducing the obscuring effects of blood and inflammation and to offer the possibility making more than one slide with homogenized representative material useful for immunocytochemistry and molecular cytology [*Chung S. et al., 1997*].

This study discusses the potential role of DNA ploidy measurement in the ThinPrep smears as an indicator of diagnostic accuracy improvement in investigation of patients with borderline and malignant common epithelial tumours of the ovary in Ia – IIc stages.

Material and Methods

Our study was carried out in 28 patients with borderline and malignant epithelial tumours of the ovary in Ia to IIc stages. All patients were investigated and operated in the 1st Department of Obstetrics and Gynecology of the National University of Athens. The age of our patients ranged from 22 to 70 years (mean value =45.2; SD=18.35). Analytically the ovarian cancers consisted of 17 serous cystadenocarcinomas, 6 mucinous cystadenocarcinomas, 2 clear-cell carcinomas, and 3 endometrioid carcinomas.

Ascetic fluids, free peritoneal fluids, and peritoneal washings were examined cytologically. For the diagnosis, WHO classification scheme was used. The comparison of histological type with the staging is presented in Table 1.

For ThinPrep cytology two smears (1 stained by Papanikolaou and 1 by Feulgen) were prepared. Both TP and conventional smears were diagnosed by cytopathologists. All negative diagnoses established by both methods, were reviewed independently by at least two cytopathologists without knowledge of the previous cytological diagnosis.

In the Feulgen-stained smears DNA ploidy measurements were performed using SAMBA 2004 Image Analyzer System (Alkatel™, Grenoble,

France) according to the standard protocol, using a Zeiss Axioplan microscope (Göttingen, Germany) with a 40:1 planachromatic lense, 3-color CCD camera, and Compaq personal computer. In each case (slide), at least 200 randomly selected nuclei were measured. Also, DNA measurements were performed in histological preparative material after removing the tumour. The estimation of DNA ploidy was based on the following parameters: DNA index, ploidy balance, degree of aneuploidy, degree of hyperploidy and proliferation index. The DNA ploidy results, final diagnosis, and stage of disease were compared.

Table 1.

Stages and Histological Types

	Stages					
Histological types	**Ia**	**Ib**	**Ic**	**IIa**	**IIb**	**IIc**
Serous	7	2	3	0	2	3
Mucinous	4	1	0	0	1	0
Clear-cell	0	0	0	0	0	2
Endometrioid	1	0	0	0	0	2
Summary	**12**	**3**	**3**	**0**	**3**	**7**

Results

For the purposes of this study, the neoplasms were divided in euploid and aneuploid. Euploid neoplasms were those with DNA value from 0.9 to 1.1 and/or from 1.8 to 2.2, while hyperploid neoplasms were those with degree of hyperploidy lower than 3. The remaining cases were considered aneuploid. Seven cases (25 %) were aneuploid and the remaining 21 cases (75%) were euploid. In our material 12 cases were borderline malignancies.

In 3 cases of stage IIc, with false-negative cytological result, aneuploid DNA was detected in the histogram of static cytometry in cytological material as well as in histological material. In 4 other cases of stage IIc, with positive cytological result, aneuploid DNA was detected as well. A statistically significant difference was observed between the final cytological diagnosis and the ploidy status for discrimination of malignant from mesothelial cell (x^2=11.25; $p<0.001$).

The sensitivity, specificity, predictive value of positive result, predictive value of negative result and the diagnostic accuracy of the final cytological diagnosis, histological diagnosis and DNA ploidy analysis were 97.62%, 100%, 100%, 91.3% and 98.09%, respectively. The comparison of the stages with DNA ploidy status is presented in Table 2.

Discussion

The high mortality from ovarian carcinoma is due to its late clinical appearance, a clinical challenge as it is often asymptomatic until the development of metastatic disease. The two thirds of patients are diagnosed, when the ovarian cancer has already reached stage III or IV. However, for early detection of disease and the correct management choice it is necessary to have a high diagnostic accuracy and prognostic factors, which may offer additional insights in early stages of epithelial ovarian cancer.

The value of tumour markers and ultrasonography to screen for epithelial ovarian cancer has not been clearly established by prospective studies. However, given the false-positive results for CA125 marker, it should not be used routinely to screen for ovarian cancer.

A hypothesis suggested that ovarian cancer was a polyclonal disease arising at multiple sites, which shared a susceptibility to events leading to malignant transformation. Few data are available about the DNA content in case of borderline and malignant epithelial ovarian tumours. Some scientists showed that there was a high correlation between FIGO stage and ploidy. The mentioned study of W. Gajewski and co-authors was aimed

Table 2.

Comparison of FIGO stages and DNA ploidy results

Ploidy	**Stage**		
	Ia- IIb	**IIc**	**Summary**
Diploid	21	0	21
Aneuploid	0	7	7
Summary	21	7	28

$p<0.001$; $\varphi=0.62$

to reveal whether DNA ploidy analysis has prognostic value in early-stage disease and whether the technique might help to identify those patients at significantly higher risk [*Gajewski W. et al., 1994*]. Based on two studies performed by J. Kaern and co-authors with matched controls they reported in one case that DNA aneuploidy was associated with an adverse clinical outcome, whereas in the other study it was not [*Kaern J. et al. 1990; 1994*]. Several studies examining benign tumours have reported that DNA aneuploidy may occur, but it is a rare finding [*Lai C. et a*l., 1996].

The purpose of our study is evaluation of liquid-based cytology for investigation of peritoneal dissemination in patients with ovarian epithelial cancer and the potential role of the DNA ploidy as an indicator of diagnostic accuracy improvement in case of epithelial ovarian cancer. Recently, attention has been focused on the correlation of diagnostic accuracy in borderline and malignant epithelial ovarian tumours with stages Ia-IIc and prognostic value of DNA ploidy. In our material, all borderline tumours were diploid.

Nine cases of stages Ib -IIb were euploid. It is important to note that surgery is the cornerstone of treatment for early-stage ovarian tumour of low malignant potential; most patients have not received postoperative adjuvant therapy and 5-year survival ranged from 90% to 100% [*Chambers J. et al*., *1988; 1989*]. Our patients (n=21) with diploid status of DNA had a good response to therapy. Seven aneuploid cases were surgically and histologically diagnosed as malignant epithelial ovarian tumour in IIc stage; respectively, they should have positive cytology in smears. However, in 3 aneuploidy cases of stage IIc the cytological findings in smears were false-negative. All patients with aneuploidy received adjuvant chemotherapy after operation. In 2 of them, metastases in pelvic cavity were detected 2.6 years later and in 1 patient metastases were detected 3.4 years later.

Based on results of our investigation, we can conclude that diploid tumours have better prognostic value as compared with aneuploidy tumours. After the review and comparison of cytological diagnosis and ploidy status, a statistically significant difference was observed between the final cytological diagnosis and the ploidy status ($p<0.001$). DNA ploidy can be measured in ThinPrep cytological smears and can help to improve the diagnostic accuracy of cytological examination, especially in cases of early stages of epithelial ovarian tumours.

Conclusion

From this study, we can report that ThinPrep is a new cytological method, which can permit a DNA ploidy measurement and will help to improve the diagnostic accuracy of cytological examination. This study showed that flow cytometric analysis of cellular DNA content acts as a useful adjunct to differential diagnosis of borderline and malignant epithelial ovarian tumours. Improving the diagnostic accuracy in early stages could help to choose the correct management for these patients. The DNA ploidy investigation dictates the necessity to determine better prognostic factors for estimation and improvement of survival in these patients.

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