



COMPARISON OF QUALITY BETWEEN CONVENTIONAL AND THIN- PREP CYTOLOGY IN INVESTIGATION OF PATIENTS WITH EPITHE- LIAL OVARIAN CANCER

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Abstract

The liquid-based cytology (ThinPrep) is an alternative to the long-used conventional cytology that received Food and Drug Administration approval in 1996. The purpose of the current study was to determine the diagnostic cytomorphologic criteria of ThinPrep cytology and a comparison of the accuracy of conventional and ThinPrep cytology in investigation of patients with epithelial ovarian cancer. We investigated the advantages of liquid-based cytology and the potential role of the DNA ploidy in investigation of patients with the ovarian cancer.

One hundred and five consecutive body cavity fluids (32 ascitic fluids, 42 free peritoneal fluids and 31 peritoneal washings) were diagnosed cytologically by both the conventional and the ThinPrep methods. The differences between ThinPrep and conventional cytology were evaluated for a variety of parameters including cellularity, cytologic morphology, specimen preparation, screening time, cytologist preference, and the impact on final diagnosis. Consequently, DNA ploidy analysis was performed on the ThinPrep specimens. The estimation of DNA ploidy was based on such parameters as the DNA index, the ploidy balance, the degree of aneuploidy, the degree of hyperploidy and the proliferation index .

According to our findings, results through the TP method were considerably improved compared with those of the conventional method. Sensitivity was improved to 90.47%, negative predictive value to 72.41% and diagnostic accuracy to 92.37%. Furthermore, these findings were in excellent correlation with the final clinical diagnosis ($p > 0.05$). According to the results of DNA ploidy analysis, 64 cases were aneuploids (60.95%), while 41 cases were euploids (39.04%). Furthermore, with the addition of the DNA ploidy analysis in our material, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were improved to 97.62%, 100%, 100%, 91.3% and 98.09%, respectively.

ThinPrep method was found to provide better cytological details, significantly improved the diagnostic accuracy of the cytological diagnosis of ovarian cancer, reduced the screening time, and permitted the valuable application of current techniques of static DNA cytometry. Furthermore, the additional application of DNA ploidy further improved the diagnostic accuracy, confirming the fact that there are changes in submicroscopical level, which are difficult to be recognized with the simple microscopical examination. Based on these findings of automatic cytology, it is possible to improve our diagnostic criteria.

Keywords: Epithelial ovarian cancer, ThinPrep, DNA ploidy.

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Introduction

The epithelial ovarian carcinoma is a relatively frequent cancer in women and the main reason of death caused by gynecological cancer [Berek J., 2000]. The high mortality from ovarian carcinoma is due to its late clinical appearance, a clinical challenge is as follows: it is often asymptomatic until the appearance of metastatic disease. The two thirds of patients are usually diagnosed, when the ovarian cancer is already at stage III or IV.

Microscopic peritoneal seeding with tumor cells predates the formation of malignant ascites. The early detection of malignant cells by free peritoneal fluid or peritoneal washing cytology may provide valuable staging and determination of prognosis.

The technique of intraoperative peritoneal washing cytology was introduced in 1956 by W. Keetle and H. Elkin [Keetle W., Elkin H., 1956]. The first systematic approach of this subject was referred by W. Keetle and N. Pixley [Keetel W., Pixley N., 1958], although Papanicolaou had already mentioned the possibility of recognising malignant cells in ascitic fluids [DeMay R., 1995]. In 1975, FIGO incorporated diagnoses of peritoneal washings into the staging classification for ovarian carcinoma.

The application of liquid-based cytology and especially the ThinPrep method in gynaecological smears started in mid 1990's both in USA and Europe and later this method was applied in non gynaecological material which was examined in cytological laboratories [Hutchinson M. et al., 1991; 1992; Awen C. et al., 1994; Perez-Reyes N.

et al., 1994; Wilbur D. et al., 1994; Biscotti C. et al., 1995; Hees K., Lebeau P., 1995].

The ThinPrep (TP) Processor (Cytoc Co., Marlborough, MA) is a thin layer preparation device that has been gaining in popularity recently. It is used to prepare ThinPrep® slides from cell suspensions collected in a preservative liquid (Cytolyt® solution). The cell suspension is at first gently dispersed, homogenizing the cell population.

The purpose of this study is comparison of quality between liquid-based cytology with ThinPrep® technique and the conventional method, in the investigation of patients with malignant common epithelial tumors of the ovary. Consequently, the potential role of DNA ploidy as an indicator of improvement of diagnostic accuracy for the ovarian cancers is investigated.

Material and Methods

Our study was carried out in 105 consecutive body cavity fluids of women hospitalized in the 1st Gynecological department of Alexandra Hospital, Medical School of National University of Athens for ovarian cancer. All patients were operated and underwent to the staging laparotomy.

The histological types with the staging of neoplasm are presented in Table 1.

The patients were from 22 to 70 years old with a mean age of 45.2 years (SD=18.35 years).

A total of 32 ascitic fluids, 42 free peritoneal fluids detected during the surgery and 31 peritoneal washings were examined cytologically. For the diagnosis, WHO classification scheme was used.

Initially, each sample of the body cavity fluid was stirred mechanically, thus achieving the

Table 1.

The histological type with the staging of neoplasms

Histological Type	Staging										
	Ia	Ib	Ic	IIa	IIb	IIc	IIIa	IIIb	IIIc	IV	Total
Serous	7	2	3	0	2	3	0	5	47	10	79
Mucinous	4	1	0	0	1	0	1	0	4	3	14
Clear-Cell	0	0	0	0	0	2	0	0	1	1	4
Endometrioid	1	0	0	0	0	2	0	0	1	0	4
Mucinous-papillary	0	0	0	0	0	0	0	0	1	0	1
Clear-cell-papillary	0	0	0	0	0	0	0	0	1	0	1
Clear-cell- serous	0	0	0	0	0	0	0	0	1	1	2
Total	12	3	3	0	3	7	1	5	56	15	105

homogeneity of the sample. For the conventional method, the samples were initially centrifuged and the supernatant fluid was discarded. Consequently, three slides were prepared by the pellet of the fluid. From these slides, two were stained by Papanicolaou stain and one by Giemsa. From the remaining material of the pellet, two additional slides were prepared by Cytospin. One was stained by Papanicolaou stain and the other by Giemsa one. In the cases of hemorrhagic fluids, the sample was resuspended in Carnoy Solution and consequently was fixed. Overall, five slides were prepared by the conventional method for each case. The three slides were stained by Papanicolaou stain (two direct smears and one cytospin) and the other two by Giemsa stain (one direct smear and one cytospin).

For the ThinPrep® method, the fluid was initially centrifuged. Consequently the supernatant fluid was discarded and the pellet was suspended in the fixative solution (Cytolyt®, Cytoc, Co, Boxborough, MA). The sample in Cytolyt® solution was centrifuged again and resuspended in cytopreservative solution (PreservCyt® solution) which mildly fixed the cells within 10-15 minutes and then the material was ready to be prepared by the ThinPrep Automated Slide Processor. In cases of bloody samples, additional Cytolyt® solution washes were necessary, until the sample became clear. Finally, for each case, two ThinPrep® slides were prepared. The one was stained by Papanicolaou stain for cytological diagnosis and the other was stained by Feulgen stain suitable for DNA ploidy.

In the Feulgen stained cytological smears DNA ploidy measurements were performed using SAMBA 2005 Image Analysis System according to the standard protocol.

Both ThinPrep and conventional smears were diagnosed by cytopathologists. All negative diag-

noses, by both methods, were reviewed independently by at least two cytopathologists who had no knowledge of the previous cytological diagnoses.

The final cytological diagnoses were provided following the review of the negative ones and were classified as, within normal limits (WNL), malignant and insufficient material (no cellular material other than blood).

Slides by both methods were rated microscopically for the following cytomorphological characteristics: degree of cellularity, presence of diagnostic cells, preservation of architecture, presence of material in the background which obscures the diagnosis and quality of staining.

Statistical analysis was performed using the McNemar test for the correlation of the results by the conventional method and the ThinPrep one. Furthermore, the Wilcoxon Signet Rank test was used for the comparison of the cytomorphological characteristics between the two methods.

The estimation of DNA ploidy was based on the following parameters: DNA index, ploidy balance, degree of aneuploidy, degree of hyperploidy and proliferation index.

Results

Our material included 110 cases of body cavity fluids. From the total number of the cases five were excluded for the purposes of this study, as the material was insufficient for cytological diagnosis by both methods.

Of 105 cases 21 were ovarian cancers stage Ia-IIb, where the presence of tumour cells in the cytological diagnosis was not expected and the 84 remaining cases corresponded to ovarian cancers stage IIc-IV.

According to our results ThinPrep smears presented a higher cellularity, a preservation of more diagnostic cells, a better architectural structure, a stain reaction and a clearer background

Table 2.

Comparison of quality between conventional and ThinPrep cytology

	Superior ThinPrep	Superior conventional	Equivalent	X ²	P
Cellularity	57	10	43	30.95	<0.001
Presence of diagnostic cells	55	10	45	29.74	<0.001
Architecture	64	11	35	37.28	<0.001
Obscuring background	13	76	21	62.60	<0.001
Stain reaction	65	17	28	33.26	<0.001

Table 3.

Correlation of the cytomorphologic characteristics between the two methods

	Poor	Fair	Excellent	Z	P
NUCLEAR DETAILS					
ThinPrep	11	48	51	-9.48	<0.001
Conventional cytology	58	45	7		
CYTOPLASMIC DETAILS					
ThinPrep	12	53	45	-8.72	<0.001
Conventional cytology	55	44	11		

than the conventional smears. Furthermore, as presented in Table 2. the presence of nuclear details, including chromatin patterns and nucleoli, was enhanced in ThinPrep smears. The cytoplasmic details were also enhanced in ThinPrep smears, there was a statistically significant difference regarding the nuclear and cytoplasmic details between the two methods ($p < 0.001$) Table 3.

Through the conventional method, false positive results were not observed, whereas in 15 of 84 cases which were expected to be cytologically positive, tumour cells were not identified in the smears. Based on these results, sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of diagnosis by the conventional method were 82.14%, 100%, 100%, 58.33% and 85.71%, respectively.

According to the TP method, false positive results were not observed either, whereas in 8 of 84 cases which were expected to be cytologically positive, tumour cells were not identified in the smears. Based on these data, sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of diagnosis by ThinPrep method were 90.47%, 100%, 100%, 72.41% and 92.37% respectively.

Out of the eight false negative cases diagnosed by the TP method, the four cases were correctly diagnosed as positive by the conventional method. Finally, according to the final cytological diagnosis, only four cases were the real false negative by both TP and conventional method. Based on these data sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of final cytological diagnosis of our material were 95.24%, 100%, 100%, 84% and 96.19% respectively. Statistically no significant difference was observed between the final cytological diagnosis and the final clinical one ($\chi^2 = 2.25$,

$p > 0.05$), whereas a statistically significant difference was observed between the conventional cytological diagnosis and the final clinical one ($\chi^2 = 64.32$, $p < 0.001$).

On the contrary, no statistically significant difference was observed between the ThinPrep cytological diagnosis and the final clinical one ($\chi^2 = 3.27$, $p > 0.05$).

The comparison of the diagnosis between the conventional and the ThinPrep method is presented in Table 4 ($\chi^2 = 10.35$, $p < 0.05$).

DNA ploidy measurements were performed and 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 aneuploid. According to our results, 64 (60.95%) cases were aneuploids, while 41 (39.04%) cases were euploids.

Finally, in Table 5 the correlation between the final cytological diagnosis, including the DNA ploidy results, with the final clinical one is presented ($\chi^2 = 2.02$, $p > 0.05$).

Based on these data, sensitivity, specificity,

Table 4.

Correlation between conventional and ThinPrep cytology

		CONVENTIONAL CYTOLOGY		
		Negative	Positive	TOTAL
THINPREP CYTOLOGY	Negative	25	4	29
	Positive	11	65	76
	TOTAL	36	69	105

$\chi^2 = 10.35$ DF=1 $p < 0.05$

Table 5.
Correlation between the cytological diagnosis adding the DNA ploidy and the clinical one

Cytological diagnosis and DNA ploidy	Staging		
	Ia – IIb*	IIc - IV	TOTAL
Negative	21	2	23
Positive	0	82	82
TOTAL	21	84	105

$\chi^2 = 2.02, p > 0.05$

* At the stage Ic it is possible to detect the malignant cells in the specimen of ascetic fluid, free peritoneal fluid or in peritoneal washing. In our material tumor malignant cells were not observed at stage Ic.

positive predictive value, negative predictive value and diagnostic accuracy adding the DNA ploidy in our material were 97.62%, 100%, 100%, 91.3% and 98.09% respectively.

Discussion

The lack of effective screening methods dictates the need of determination of better prognostic factors for estimation of the survival of patients with epithelial ovarian cancer [Omura G. et al., 1991; Berek J.S., 2000]. Thus, the cytological examination of the ascitic fluid, of the free peritoneal fluid and of the peritoneal washing is a well-accepted method for the investigation of patients with epithelial ovarian cancer [Youshimura S. et al., 1984].

In 1975 the International Federation of Gynaecologists and Obstetricians (FIGO) incorporated results of peritoneal washing cytology into the staging classification for ovarian carcinomas [FIGO, 1986; 1998].

Consequently different cytopreparatory techniques have been developed. Morton (1961) and Graham (1962-1967) evaluated the cytological diagnosis for the early diagnosis of metastatic disease, applying pioneering techniques, such as peritoneal washings and transvaginal aspirations of Douglas cul-de-sac. These techniques became known to Europe by the reports of J. Dupre-Froment and J. De Brux in the mid 1960's [Dupre-Froment J. et al., 1967; De Brux J. et al., 1968].

The cytological evaluation of effusions and especially peritoneal washings and ascitic fluids obtained during the surgery, is quite difficult and requires experienced cytopathologists. For this reason, the cytological examination was not widely applied

in the investigation of malignant ovarian tumours, until it was incorporated in the staging of FIGO protocols [FIGO, 1986; 1998].

The adequacy of the smear is another important issue in the evaluation of cytological specimens. From 1970 until nowadays, 3000 cases have been reported in the literature without reference to insufficient smears which is characteristic of absence of criteria. Although there are some cases with the cytological diagnosis “no malignant cells are observed” it would be more appropriate if those cases were referred like “cytological elements are not observed” [Youshimura S. et al., 1984].

In our material all five cases with insufficient material by both methods were observed only in peritoneal washings specimens.

In previous studies, the diagnostic accuracy for the conventional cytology ranged from 54% to 96%.¹³ However, several investigators reported false negative cytological diagnoses of ovarian cancers, after histological confirmation of the peritoneum dissemination, to be ranged from 20% to 70%, and the most of those were concerned peritoneal washings [Youshimura S. et al., 1984; Pretorius R. et al., 1986; Rubin S. et al., 1988].

According to our findings, 15 false negative cases and none false positive were observed in the conventional slides. These findings prove that the conventional method is reliable, as the diagnostic accuracy was 85.71% and the positive predictive value was 100%, but an important problem arise regarding the evaluation of negative results, as the negative predictive value was 58.33%. The negative predictive value is affected by the fact that out of the 10 cases with peritoneal metastases but without the presence of ascitic fluid or free peritoneal fluid during the surgery, only in 3 cases neoplastic cells were detected by peritoneal washings. This is due to the low cellularity of the observed material, as the smears were bloody, so the background obscured the diagnostic cells.

Five peritoneal washings with insufficient material and 7 false negative cases out of the 10 with peritoneal metastases were observed, but without the presence of ascitic or free peritoneal fluid during the surgery. The remaining false negative cases consisted of 6 free peritoneal fluids out of the total of 42 cases and 2 ascitic fluids out of the total of 32.

The purpose of this study is the comparison of

liquid-based cytology with conventional cytology in the investigation of peritoneal dissemination to patients with ovarian epithelial cancer and the potential role of the DNA ploidy as an indicator of improvement of diagnostic accuracy of the ovarian cancers.

It has been proven in gynaecological smears, that the ThinPrep method permits the concentration and immediate fixation of the examined specimens and its homogenization, thus making possible the Thin preparation of more slides with the same representative cellular material. Furthermore, the nuclear and cytoplasmic details are better preserved than those of the conventional method.

In addition, through the ThinPrep method, the cytological diagnosis is possible in one only slide, while in the conventional method at least five slides were necessary.

The improvement of the quality of cytomorphological characteristics in the ThinPrep method results in the reduction of false negative rate in 8 cases without the presence of any false positive result. This results in a statistically significant difference of the diagnostic accuracy between the two methods ($\chi^2=10.35$, $p<0.05$).

Furthermore, sensitivity improved from 82.14% to 90.47%, negative predictive value from 58.33% to 72.43% and, finally, the diagnostic accuracy improved from 85.71% to 92.37%. These findings are in excellent correlation with the final clinical diagnosis ($\chi^2=3.27$, $p>0.05$).

Furthermore, the final cytological diagnosis resulted in the reduction of false negative diagnoses in 4 peritoneal washings consisting by one serous papillary adenocarcinoma stage IIIc, one serous papillary adenocarcinoma stage IIc and two serous papillary adenocarcinoma stage IIIc. The correlation of the final cytological diagnosis with the final clinical one did not show a statistically significant difference ($\chi^2= 2.25$, $p>0.05$).

Based on these findings it appears that despite its additional cost, the ThinPrep method improves the diagnostic accuracy of the cytological diagnosis. We were initially cautious of using this new cytopreparatory technique, since it added substantial cost to already existing cytodiagnostic procedures, because of high cost of the reagents. Nevertheless, the ThinPrep method offers certain advantages over the conventional method, the most important being the rapid screening, through the

reduction of the time of microscopy at least to 1/5, as it needs only one slide for the diagnosis in comparison with the five used by the conventional method. Furthermore, the ThinPrep method allows the creation of more slides with representative cellular material for the purposes of immunocytochemistry and molecular cytology.

In our material, the DNA ploidy analysis was performed using image analysis system. According to our findings, even in cases of the euploid neoplasms it is possible to find metastasis in the peritoneal cavity and this fact proves that it is impossible to use the nuclear DNA content alone as a marker for the presence of tumor cells.

However, two cases, in which the cells did not present clear characteristics of malignity, were recognised as aneuploid neoplasms reducing in that way the false negative results from 4 to 2.

In spite of the fact of high diagnostic accuracy, based on cytomorphologic criteria of our department, the further improvement adding the DNA ploidy was not statistically significant ($\chi^2=2.02$, $p>0.05$).

More specifically, in the two peritoneal washings from the four cases with false negative diagnoses by both methods, clones of aneuploidy in the histograms of static DNA cytometry were recognised. Based on the above, the DNA ploidy analysis added to the ThinPrep cytological diagnosis could have reduced the unnecessary diagnostic procedures for the patient.

Moreover, another study is developing with the follow-up of patients to conclude if the cytological study of the body cavity fluids with systems of static DNA cytometry was in correlation with the prognosis.

In summary, the liquid-based cytology improved significantly the diagnostic accuracy of the cytological diagnosis, reduced the screening time and permitted the valuable application of current techniques of static DNA cytometry.

In spite of the fact that further reduction of the false negative diagnoses is not possible to improve the diagnostic accuracy significantly, the application of the static DNA cytometry confirms the fact that there are changes in submicroscopical level which are difficult to be recognized with the simple microscopical examination. Based on the findings of automatic cytology, it is possible to improve our diagnostic criteria.

References

1. *Berek J.S.* Epithelial ovarian cancer. In: Practical Gynaecologic Oncology. Edited by Berek J.S., Hacker N.F. 3rd edn. Philadelphia, Lippincott Williams & Wilkins. 2000. P. 457-522.
2. *Keetle W.C., Elkin H.B.* Experience with radioactive colloidal gold in the treatment of ovarian carcinoma. *Am. J. Obstet. Gynecol.* 1956; 71: 553-568.
3. *Keetel W.C., Pixley N.N.* Diagnostic value of peritoneal washings. *Clin. Obstet. Gynecol.* 1958; 1: 592-606.
4. *DeMay R.* The art and Science of Cytopathology, Exfoliative Cytology. HSCP Press, Chicago. 1995. P.1.
5. *Biscotti C.V., Hollow J.A., Toddy S.M., Easley K.A.* ThinPrep versus conventional smear cytologic preparations in the analysis of thyroid fine-needle aspirations specimens. *Am. J. Clin. Pathol.* 1995; 104: 150-153.
6. *Hees K., Lebeau P.B.* Comparison of conventional and ThinPrep preparations of mucoid cytology samples. *Diagn. Cytopathol.* 1995; 12: 181-185.
7. *Perez-Reyes N., Mulford D.K., Rutkowski M.A., Logan-Young W., Dawson A.E.* Breast fine-needle aspiration. A comparison of thin-layer and conventional preparation. *Am. J. Clin. Pathol.* 1994; 102: 349-353.
8. *Awen C., Hathways S., Eddy W., Voskuil R., Janes C.* Efficacy of ThinPrep preparation of cervical smears: a 1.000 case, investigator-sponsored study. *Diagn. Cytopathol.* 1994; 11: 33-36.
9. *Hutchinson M.I., Cassin C.M., Ball H.G.* The efficacy of an automated preparation device for cervical cytology. *Am. J. Clin. Pathol.* 1991; 96: 300-305.
10. *Hutchinson M.I., Agarwal P., Denault T., Berger B., Cibras E.S.* A new look at cervical cytology: ThinPrep multicenter trial results. *Acta Cytol.* 1992; 36: 499-504.
11. *Wilbur D.C., Cibas E.S., Merritt S. et al.* ThinPrep Processor. Clinical trials demonstrate an increased detection rate of abnormal cervical cytologic specimens. *Am. J. Clin. Pathol.* 1994; 101: 209-214.
12. *Omura G.A., Brady M.F., Homesley H.D. et al.* Long term follow-up and prognostic factor analysis in advanced ovarian carcinoma: the Gynecologic Oncology Group experience. *J. Clin. Oncol.* 1991; 9: 1138-1350.
13. *Youshimura S., Scully R.E., Taft P.D., Kerrington J.B.* Peritoneal fluid cytology in patient with ovarial cancer. *Gynecol. Oncol.* 1984; 17: 161-167.
14. *De Brux J., Dupré-Froment J., Mintz M.* Cytology of the peritoneal fluids sampled by coelioscopy or by cul-de-sac puncture. Its value in gynecology. *Acta Cytol.* 1968; 12(5): 395-403.
15. *Dupré-Froment J., Mintz M., De Brux J.* Cytologie peritonealle par prélèvements per coelioscopiques ou par ponction du Douglas. *Gynecol. Obstet.* 1967; 66 (1): 95-108.
16. *FIGO Cancer Committee.* Staging Announcement. *Gynecol. Oncol.* 1986; 25: 383.
17. *FIGO Committee of Cynecology Oncology:* FIGO annual report on the results of treatment in gynaecologic cancer. *J. Epiemiol. Biostat.* 1998.
18. *Pretorius R.G., Lee K.R., Papillo J., Baker S., Belinson J.* False-negative peritoneal cytology in metastatic ovarian carcinoma. *Obstet. Gynecol.* 1986; 68: 619-623.
19. *Rubin S.C., Dulaney E.D., Markman M. et al.* Peritoneal cytology as an indicator of disease in patients with residual ovarian carcinoma. *Obstet. Gynecol.* 1988; 71: 851-853.