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Does Cytological Laboratory Holds the Responsibility for the Low Sensitivity of the PAP Test in Detecting Endometrial Cancer?

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ABSTRACT

Endometrial cancer is the most common gynecological cancer but there is no economically justified screening method. Although we can detect endometrial cells in the sample using PAP test, many studies show low sensitivity and positive predictive value of PAP test for the diagnosis of endometrial cancer. The goal of this research was to determine significance of PAP test for the diagnostics of endometrial carcinoma. Sensitivity and specificity were analyzed with statistical parameters. VCE (vaginal, cervical, endocervical) smears of patients with histologically proven endometrial carcinoma were re-examined in order to determine the proportion of false negative results for endometrial cancer cells in the VCE samples. Study group consisted of all consecutive patients with PAP test performed at the Department of Clinical Cytology of the University Hospital Center Osijek from 2002 until the end of 2014. There was one inclusion criteria: subsequent hysterectomy or curettage within the six month after the PAP test, regardless of histological finding. From a total of 263 patients with previous PAP test and histologically proven endometrial cancer, endometrial cancer was cytologicaly diagnosed in 24.7% (including suspicious and positive findings), while 66.2% patients had normal cytological findings. The diagnostic value of PAP test in detection of endometrial cancer was statistically revealed with 25% sensitivity and 99% specificity. To determine false negative rate VCE samples were reviewed for patients with histologically proven endometrial cancer and negative VCE findings. There were a total of five negative results. In one case revision did not changed the original negative diagnosis, but benign endometrial cells, a lot of blood and inadequate cytohormonal status were found. In three out of four reviewed samples there were missed cells of endometrial adenocarcinoma. Review of remaining VCE sample upgraded the diagnosis from negative to suspicious for endometrial cancer. Proportion of error in the detection of endometrial cancer using cytological findings was 3.4% (true false negatives). Negative rate of the cytological findings in the detection of endometrial cancer was 66.2%. PAP test is not a suitable method for detection of endometrial carcinoma due to low sensitivity (25%). The main cause of negative findings in PAP test was lack of diagnostic cells in the sample.

Key words: *cytology, cell biology, histology, PAP test, endometrium, neoplasm, VCE sample, sensitivity, specificity, screening*

Introduction

Endometrial cancer is the most common malignancy of the female genital tract[1]. Unlike cervical cancer, whose incidence has significantly dropped due to implementation of opportunistic and organized screening, there is no economically feasible screening test for endometrial cancer[2].

The incidence of endometrial cancer is constantly increasing. The increase is accelerated by prolonged life expectancy, certain predisposing factors, but also the improvement of diagnostic procedures and a consequent improvement of the cancer detection[3]. Considering the fact that the increase in incidence is particularly pronounced in the highly developed countries[1] where cervical screen-

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ings are implemented, there is a need to investigate the possibility of using Pap test in the diagnosis of endometrial cancer.

Participants and Methods

This is a retrospective cross-sectional comparative study. The study included patients that had a cytological examination of VCE smear at the Clinical Department of Clinical Cytology, University Hospital Center Osijek during the period from 2002 to the end of 2014 provided they had a hysterectomy or fractionated curettage of the endometrium during a period of six months after the diagnosis. Cytology samples were fixed in 95% alcohol and stained by the method of Papanicolaou. Taking into account the criteria for differential cytology, classification Zagreb 2002[4], which represents a Croatian modification of the Bethesda classification (TBS 2001)[5] was used for cytology diagnosis. Histologic examinations, were performed at the Clinical Department of Pathology and Forensic Medicine, Clinical Hospital Centre Osijek. Histological diagnosis was performed on samples obtained by fractional curettage or resection material, fixed in formaldehyde, embedded in paraffin cubes and described according to current WHO classification[6].

Age at diagnosis, cytological diagnosis and the most severe histological diagnosis were determined for each patient. Cytological diagnoses were divided into three categories: benign lesions, lesions on the cervix, and lesions that suggest endometrial pathology (atypical glandular cells where invasive lesion (AGC-I) (endometrial), adenocarcinoma cannot be excluded). Samples that comply with the criteria were rescreened in order to determine the weak points of the screening.

Statistics

Descriptive statistical methods were used for a description of the studied variables and frequency distributions. All variables were tested for normal distribution using the Kolmogorov-Smirnov test and depending on the result non-parametric methods were applied. Mean values of continuous variables are expressed as medians as well as range for the variables without normal distribution. Categorical variables are presented by the frequency distribution within group and percentage share. Mann Whitney test was used to determine if there is statistically significant difference in age between patients with different cytological diagnoses. Fisher's exact test was used to determine the difference between the rations. Significance level $\alpha=0.05$ was chosen as the significance threshold. All p values were bilaterally. Statistical analysis was used statistical program SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) was used for all the statistical analyses. The diagnostic value of cytology for the detection of malignancy (cancer) of the endometrium was evaluated according to sensitivity and specificity.

Results

The study included 5105 patients who complied with the inclusion criteria. In most of the patients (5007 cases (98.1%)), VCE smear did not reveal any morphological changes that comply with the criteria for a suspected outbreak or definite diagnosis of endometrial malignancy, while the same was found in the cytological smear of 98 patients (1.9%). The mean age of patients was 44 years (interquartile range 33-53 years). The youngest patient was 16 years and the oldest was 91 years old. There is statistically significant difference in age between patients with suspected or proven cytological endometrial lesions

TABLE 1
THE RATIO OF CYTOLOGICAL AND THE MOST SERIOUS HISTOPATHOLOGICAL DIAGNOSIS (N=5105)

Cytological diagnosis		Histopathological diagnosis					Total	p‡
		LOC*	Benign	Hyperplasia	Endometrial ca.	Other malignant neoplasms		
LOC*	N	2690 (88.3%)	324 (10.6%)	5 (0.2%)	24 (0.8%)	3 (0.1%)	3046 (100%)‡	
	%	97.2	16.8	4	9.1	20	59.7	
Negative	N	63 (3.2%)	1598 (81.4%)	119 (6.1%)	174 (8.9%)	7 (0.4%)	1961 (100%)	<0.001
	%	2.3	82.6	94.4	66.2	46.7	38.4	
Malignant endometrial	N	14 (14.3%)	12 (12.2%)	2 (2.1%)	65 (66.3%)	5 (5.1%)	98 (100%)	
	%	0.5	0.6	1.6	24.7	33.3	1.9	
Total	N	2767	1934	126	263	15	5105	
	%	100	100	100	100	100	100	

*LOC - lesion on cervix, †Fisher's exact test, ‡percentages are only relate (in all horizontal lines) to calculation in horizontal lines

TABLE 2
THE PARAMETERS OF THE DIAGNOSTIC VALUE OF PAP TEST IN DETECTING MALIGNANCIES OF THE ENDOMETRIUM (%)

Cytological diagnosis	Total number	SE*	SP†
LOC‡	3046	97	85
Negative	1961	83	92
Malignant endometrial	98	25	99
Total	5105	88	94

*sensitivity, †specificity, ‡LOC - lesion on cervix

(they are older), mainly middle-aged with mean age 66 (interquartile range from 57 to 72.5 years) (Mann Whitney test, $p<0.001$).

Cytological diagnoses were compared to the most severe histological diagnoses. Regardless of the type of a histological sample, cytological cervical lesions were proven and histologically confirmed in 2690 (88.3%) cases, a normal cytological findings were confirmed in 1717 (87.6%) cases, while the malignant lesions of the endometrium (or suspected malignant lesions) were confirmed in 70 (71.4%) cases (Table 1). In histologically confirmed endometrial cancer (total 263 cases) Pap test was normal in 174 patients (66.2%), and in 65 patients (24.7%) endometrial cancer was diagnosed or suspected.

Cytological diagnosis of cervical lesions was histologically proven as malignant endometrial lesions in 27 cases (0.9%), while in 119 cases (6.1%) normal cytological findings corresponded to endometrial hyperplasia, in 174 cases (8.9%) endometrial cancer, and in 7 cases (0.4%) some other malignant neoplasm of the endometrium.

Comparing the most severe cytological diagnoses with the most difficult histological diagnoses, regardless of the type of histological samples (Table 1), the cytological diagnosis were classified as true positive, false positive, true negative and false negative. Based on those categories, parameters of differential diagnostic value of cytological diagnosis were calculated (Table 2).

Sensitivity of cytological diagnosis (Pap test) in the detection of endometrial malignancy is 25%, while the specificity is high (99%) (Table 2).

If only endometrial carcinomas were considered, values are similar, which is a result of the largest ratio of endometrial cancer in the total number of cancer lesions.

A total of 181 samples were re-screened (174 histologically proven endometrial cancer and 7 of other malignant neoplasms of endometrium) and five negative cytological findings (2.8%) were revised. In three cases there was missed diagnosis of endometrial adenocarcinoma. In one case, the diagnosis was revised to a suspicion of endometrial cancer (AGC - cannot exclude invasion (endometrial)), and in one case diagnostic cells were not found, but the clinical status revealed high hormone level that did not correspond to the age or medical history, plenty of blood and the presence of endometrial cells with normal morphological appearance which the primary screening did not diagnose (Table 3).

A ratio of errors in cytology findings in detecting endometrial cancer is 3.5%, if calculated as the number of women who have endometrial malignancy and negative cytological findings in relation to the total number of analyzed cytological samples. The revision has revealed that only four samples of the total number with false negative cytology are not recognized by cytologists (cytotechnician), while the others contained no diagnostic cells and the proportion of errors due to cytology laboratory work is 0.08%. The error ratio for cytological findings in detection of endometrial cancer (proportion of false negative) calculated as the number of women who have endometrial malignancy and negative cytological findings with respect to all respondents with a malignant disease of the endometrium is 66.2%. Since the revision identified four false-negative cytological findings as a reflection of errors in the interpretation of cytology, ratio of false negative that are responsibility of cytology laboratory performance is 1.5%.

Discussion

In addition to its main purpose Pap test can be used to detect the presence of endometrial cells, although many studies had shown low sensitivity and positive predictive value in their detection. After implementation of Bethesda classification TBS 2001 research had shown that the detection of presence of endometrial cells with normal morphological appearance leads to greater number of endometrial tests but it did not lead to the significant advancement in detection of malignant changes of endometrium[7]. Reasons for low sensitivity in detection of endometrial carci-

TABLE 3.
REVISION RESULTS OF CYTOLOGY NEGATIVE FINDINGS FROM THE PRIMARY SCREENING

Cytological diagnosis in the first screening	Cytological diagnosis – revision				
	Benign	Endometrial cells, high hormone, plenty of blood	AGC-I*	Adenocarcinoma of the endometrium	Total
Benign	176	1	1	3	181

*AGC-I - atypical glandular cells, can not exclude invasion

noma with Pap test are mostly due to anatomical and physiological factors. Those can be separated into two possible groups, group where certain factors can affect morphology of endometrial cells and the group where factors affect the number of endometrial cells in smears. Endometrial cells morphology can be changed. Degenerative changes on cells are often due to long path of cells from uterus to vagina. Also morphologist can sometimes have difficulties in differentiation between atypical endocervical and endometrial cells. Cancer cells are diploid, rarely aneuploid, and hyperchromasia (as important criteria of malignancy) is usually not expressed. Also, exfoliation in well-differentiated carcinoma is poorly expressed. Diagnostic cells are rarely found or not at all in postmenopausal women, because in those women cervical stenosis can be found often. Cells of poorly differentiated cancer are small, and look like core of intermediate squamous cells, and they can be found in smaller number in cervicovaginal smears, and because of that other cytological parameters are important: appearance of the smear base, tumor diathesis and cytohormonal status[1,8-9].

Cytological laboratory of Clinical hospital center Osijek describes its findings of endometrial cells in VCE smears according to modification of Bethesda classification, Zagreb 2002, in three categories: morphologically benign looking endometrial cell, ASC- invasion cannot be excluded (endometrial cells) and endometrial adenocarcinoma. To evaluate the diagnostic value of Pap test in detection of endometrial carcinoma, histological findings of patients with previous hysterectomy or fractionated curettage within six months from the last VCE smear were compared.

Mean age of patients with cytologicaly suspected or proven lesion on endometrium in this study is 65 years while the mean age of patients with cytological normal findings or diagnosed cervical lesion was 43 years. Many other studies have similar results[10]. Snyderi and associates (2001) found the greatest number of lesions in group of women over 60 years of age[11]. Mean age of patients reported in study of Gu and associates (2001) that correlated Pap test with histologically proven endometrial cancer, was 65 years[12].

In 66.2% of patients with histologically proven endometrial cancer Pap test was normal, and in 24.7% was diagnosed as endometrial cancer or suspicion to endometrial cancer. Such large number of negative cytological findings is resulting with a low sensitivity of conventional Pap test in diagnostics of endometrial cancer only 25%. Sams and associates (2012) had compared conventional Pap test with liquid-based cytology and have also reported low sensitivity (20-30%). Other literature sources report sensitivity of Pap test between 40-70%[8,13-14].

In cases where cytological findings report suspicion on malignant endometrial disease or in cases of set diagnosis, the same diagnosis is hystologically proven in 71.4% of cases. In 14% of the cases cytologist has recognized the existence of pathology, but he misjudged the origin of pathologically altered cells. Only 12.2% of women with cytological diagnosed endometrial pathology have had a false positive test, which results in high specificity of cytological differential diagnosis.

Fujiware et al. (2015) have conducted research of cytohistology correlation on 1441 female patients with uterus carcinoma. In their research the specificity of cytology to detect endometrial carcinoma was 98.8% which is in accordance with results of this study[15].

Usually there are two methods to determine the ratio of errors in cytological findings. First one is to determine the number of women who have endometrial pathology and negative cytology findings in relation to the total number of cytology smears analyzed through the observed period. This method has very low proportion of error. Second method is to determine the proportion of errors by determining the number of women who have endometrial pathology and calculating percentage of normal cytology findings. This method has greater percentage of errors. Such a large difference reflects the fact that most of the cytological findings are without pathology. This opens a question, about the level of cytology ability to detect this small percentage of abnormal samples. This result is called false negative ratio, and is calculated as a number of cytology findings that are interpreted as normal in women with endometrial pathology in relation to number of women with endometrial pathology. False negative ratio in this study is extremely high (up to 66.2%). In order to determine the cause of so many false negative samples, we rescreened all false negative cytology samples in which endometrial carcinoma was proven histologically. With rescreening we identified four diagnostic failures, and it has been found that 177 samples did not contain diagnostic cells needed to determine endometrial pathology.

Conclusion

Pap test as method is not satisfactory in diagnosis of endometrial pathology (sensitivity 25%, false negative ratio 66.2%). The errors in cytotehnologist and cytologist interpretation make up a small proportion of the false negative results, indicating that the insufficiency of the method is determined primarily by anatomical and physiological factors. But, it is necessary to put special attention to the interpretation of the origin of pathological cells (because of possible confusion with the pathology of the cervix), the findings of benign endometrial cells and the presence of other cytological parameters such as appearance of background smear, tumor diathesis and cytohormonal status of woman.

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LEŽI LI ODGOVORNOST ZA NISKU OSJETLJIVOST PAPA TESTA U DETEKCIJI ENDOMETRALNIH KARCINOMA NA CITOLOŠKOM LABORATORIJU?

SAŽETAK

Za razliku od cervikalnoga karcinoma, čija je pojavnost značajno opala implementacijom oportunističkog ili organiziranog probira, za endometralni karcinom, unatoč tome što se radi o najčešćoj malignoj bolesti ženskog genitalnog trakta, ne postoji ekonomski opravdan skrining test. Obzirom da je rastuća incidencija istog osobito izražena u visokorazvijenim zemljama u kojima postoji implementiran cervikalni skrining nameće se potreba preispitati mogućnosti PAPA testa u dijagnostici endometralnih karcinoma. Presječnom retrospektivnom studijom obuhvaćene su ispitanice u kojih je na Kliničkom zavodu za kliničku citologiju Kliničkoga bolničkoga centra Osijek u razdoblju od 2002. do kraja 2014. godine izvršen citološki pregled VCE razmaza, uz uvjet da su u vremenskom intervalu od najduže šest mjeseci nakon citološke dijagnoze imale histerektomiju ili frakcioniranu kiretažu endometrija. Dijagnostička vrijednost citologije za otkrivanje karcinoma endometrija procijenjena je na temelju osjetljivosti i specifičnosti. Uzorci koji zadovoljavaju kriterije studije reskrinirani su u cilju utvrđivanja propusta u skriningu. Od 263 histološki potvrđena endometralna karcinoma prethodnim Papa testom, endometralni karcinom (odnosno sumnja na isti) dijagnosticiran je u 24,7% ispitanica, dok je u 66,2% ispitanica citološki nalaz bio uredan. Parametri dijagnostičke vrijednosti Papa testa u detekciji endometralnih karcinoma iznose: osjetljivost 25% i specifičnost 99%. Reskriningom VCE uzoraka ispitanica s histološki dokazanim karcinomom endometrija u svrhu utvrđivanja uzroka lažno negativnih nalaza revidirano je ukupno pet negativnih citoloških nalaza, od kojih kod jednog revizijom nije mijenjana prvobitno postavljena negativna dijagnoza, ali je utvrđeno postojanje benignih endometralnih stanica, obilje krvi te citohormonski status koji ne odgovara dobi ili anamnezi. Od ostala četiri uzorka u tri slučaja je dijagnosticiran propušteni adenokarcinom endometrija, a u četvrtom je postavljena sumnja na isti. Porporcija pogreške citološkog nalaza u otkrivanju karcinoma endometrija iznosi 3,4%. Lažno negativna proporcija citološkog nalaza u otkrivanju karcinoma endometrija iznosi 66,2%. Papa test zbog svoje niske osjetljivosti (25%), koja je prije svega posljedica nedostatka dijagnostičkih stanica u uzorku, nije pogodna metoda za detekciju endometralnih karcinoma.