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EFFECTIVENESS OF EVALUATION OF CERVICAL PRECANCEROUS PATHOLOGY USING *SHANDON PAPSPIN* SYSTEM

Summary of doctoral dissertation

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VILNIAUS UNIVERSITETAS

Jolita Rimienė

GIMDOS KAKLELIO IKINAVIKINĖS PATOLOGIJOS ĮVERTINIMO EFEKTYVUMAS NAUDOJANT *SHANDON PAPSPIN* SISTEMĄ

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CONTENT

ABBREVATIONS

Pap	Papanicolaou test
H&E	Hematoxylin and eosin staining methods
μm	micrometers
mm	millimeters
SD	standard deviation
ASC	atypical squamous cell
ASC-US	atypical squamous cell undeterminate significance
ASC-H	atypical squamuos cell suggestion for high-grade squamous intraepithelial lesion
LSIL	low grade squamous intraepithelial neoplasia
HSIL	high-grade squamous intraepithelial lesions
SCC	squamous cell carcinoma
AGC	atypical glandular cell
AGUS	atypical glandular cells undeterminate significance
AIS	adenocarcinoma *in situ*
AC	adenocarcinoma
NILM	negative for intraepithelial leasion
CIN	cervical intraepithelial neoplasia
CIN1	cervical intraepithelial neoplasia (Grade I)
CIN2	cervical intraepithelial neoplasia (Grade II)
CIN3	cervical intraepithelial neoplasia (Grade III)
CIS	carcinoma *in situ*
ASCCP	American society for colposcopy and cervical pathology
WHO	World Health Organization
CI	Confidence Intervals
ROC	Receiving operating curve
PPV	positive predictive value of the study
NPV	negative predictive value of the study
PCR	polymerase chain reaction
CAP	College of American pathologists
NCI	National Cancer Institute
LLETZ	Large loop electroexcision with transformation zone
ALTS	ASCUS/LSIL triage study
HPV	Human papilloma virus
PBS	Phosphate buffer solution
VUIO	Vilnius University Institute of Oncology

1. INTRODUCTION

1.1 Study problem

The cancer is actual problem of public health. For more than 10 million people worldwide are diagnosed with some form of cancer each year .

Despite particular efforts to prevent cancer the incidence of malignant neoplasms and mortality is increasing. World Health Organization predicted that the number of cancer cases by 2010 in the world will increase by another 50%, resulting in more and more attention should be paid to primary and secondary cancer prevention. Part of the cancer-causing risk factors and their prevalence in the population groups are determined by epidemiological studies, allowing for primary prevention of disease to be done. It is important to note that the developed vaccine against carcinogenic viruses complements the primary cancer prevention measures. Among secondary prevention measures are important screening examination of population health which are supposed to cover of 80 percent population .

Cervical cancer is the second most frequent malignancy worldwide after the most frequent breast cancer and the third leading women cause of death from cancer worldwide after most common breast and lungs. 471 000 new cervical cancer cases are estimated and more then 230 000 deaths occur annually around the world . Over 80 percent of cases of cervical cancer are registered in the developing countries. There are a lot of knowledge about cervical cancer development and measures to prevent this disease. Precancerous cervical lesions become cancer over a long period of time. They can be diagnosed by cytological test of cervical smears and treated thus to avoid cervical cancer. Periodic screening of clinically healthy women with use cervical cytology smears is the effective way to reduce incidence and mortality of the cervical cancer. In some countries the introduction of screening for cervical cancer significantly reduced the number of cases of this disease. The screening for cervical cancer in some part of the world (USA, Finland) is carried out about 40-30 years. Then the incidence of cervical cancer and mortality from it decreased significantly about 70-80%. Implementing of screening for cervical cancer a direct correlation between the frequency of screening and decline in the mortality has been observed. The knowledge about cervical cancer risk factors has been expanding throughout recent years. 2008 prof. H. Zur Hausen, the Nobel laureate, demonstrated the role of human papilloma virus causing cervical precancerous lesions and cancer forced to look at this disease as the spreading sexually and of infectious origin. A new weapon was developed to fight the cervical cancer - the vaccine against human papilloma virus, for immunization of girls 8-12 years old. Combining these two preventive measures - vaccination of young girls and screening of women with cervical cytological smears - 90% of cervical cancers can be avoided. Cytological cervical smear test to diagnose precancerous lesions introduced in 1928 (Papanicolaou test) to this day remains the standard screening for early detection of precancerous cervical cells lesions. The false positive and false negative results are specific for cervical cytological test. It was found that invasive cervical cancer false negative results may be received in 16-82% of cases. Low sensitivity of the investigation is the main weak part of cytological cervical smear test. It is known, that it leads to a lack of sensitivity of smear staying impurities (inflammatory exudates, blood, mucus, cellular air-drying artifacts, preparing smear deformed cells, etc.). In the cytological smear test in this conclusion has to write the cytological smear is inadequate to cytological research, or that the cells are of undeterminate categories (ASC, ASC-H). In finding ways to improve cervical cytological analysis (Pap test) systems, automatically performing cytological cervical smear tests were created. While creating automated analysis system, cervical cell fixation and transportation in liquid media way was developed. From cells fixed in liquid media made-up preparations are one layer, that allow to see the microscopic cells in a high-quality picture, with no impurities, ideally suitable for cytological investigation. It appears that such preparations more effectively investigated by humans than automatic systems. It should be emphasized

that the preparations made from the cells, fixed in liquid media, characterized by low percentage of uncertain categories (ASC, ASC-H) cells, enables to iincrease the detection of HSIL cases by 18%. A number of automatic systems of thin-layer preparations of cervical cells for cytological investigation were created soon afterward. On the basis of the new technologies used in the preparation of cervical cytological material research results in 1996 U.S. Food and Drugs Administration approved the cytological test preparation from the cervical cells, fixed in liquid medium, the membrane filtration method which, based on *ThinPrep* (Cytyc Corporation, Boxborough, MA) system later in 1999, UW TriPath Imaging, Burlington, NC confirmed *SurePath* system. Since then, were published 147 prospective scientific studies, 15 meta-analysis showing *ThinPrep* and *SurePath* diagnostic system effectiveness, their economic impact on screening program results, compared with effectiveness of the cytological cervical smear test. On the other hand, cost-benefit analysis of the new systems has shown that there is a cheaper alternative of quality systems, which for years have been effectively used in the Central European laboratories. One of them *PapSpin* (ThermoShandon Inc., Pittsburg, USA) – cytological system based on cytocentrifugation principle, which has more than 50 years is associated with requirements of good laboratory practice. It could be possible to adapt this system to investigate the cervical material and the sample of cervical screening, but there is little research that would enable an assessment of the centrifugation-based diagnostic system efficiency and impact of selective cervical screening programs.

1.2 Relevance and importance of study

A priority health area is the reduction of the incidence of cervical malignancies. The incidence of cervical cancer is the largest in Lithuania in the Baltic region and in the second place after Romania in Eastern Europe. The Government of Lithuania Republic approved the National Cancer Prevention and Control program for 2003-2010 years on the 3 December, 2003, the main objective is - to reduce the incidence of malignant tumors, mortality from these diseases, number of disabled people with oncological diseases. It was expected that screening of samples of cervical pathology will reduce the incidence of cervical cancer by 30 percent. The screening program for cervical pathology in Lithuania was introduced in July 2004. In the screening is used cytological cervical smear test. The largest part of effectiveness of screening program depends on quality of the test used. It is noted that improving the effectiveness of the test used in screening manifests in indirect affects in morbidity and mortality decrease.

It should be noted that the effectiveness of the cytological cervical smear test was not evaluated in prospective studies, however, the possibilities and advantages of early diagnosis of cervical precancerous lesions of this test were demonstrated. The cytological smear test isn't ideal, for its typical false negative and false positive test results. The average rage of cytological smear test sensitivity is 49%; average rage of the specificity is 95% . The major disadvantage of conventional smear is large number of unsatisfactory smears and uniquivocal categories. It is also often cytological smear is thick, with numerous impurities, air-dry artifacts, deformed cells. Similar defects specific for smears are identified in Lithuania in the cervical screening program as well. By The State Patient's Fund „*Sveidra*" informative system data studying the smears finding high 5-12% swabs inappropriate content, large percentage of undeterminates ASC / ASC-H diagnoses, lack of detection of HSIL cases. One of the main reasons is a lack of quality of cytological smear due to methodological shortcomings of the smear preparation. It is proved that 50% of cells from the material collection tool don't hit on a smear slide and are discarded with the instrument .

Since 1990 cervical cell cytological tests are examined using of fixed in liquid media cervical cells in U.S. World literature is rich in scientific papers analyzing the *ThinPrep* and SurePath system diagnostic accuracy as well as is demonstrated impact on the effectiveness of screening programs. About 70 percent cervical cytological tests are investigated using *ThinPrep* and *SurePath* systems worldwide. These two systems monopolizing the area of

cervical researches cytological tests of fixed cells in liquid media has become expensive. The analysis of cost-benefit has to force a search for cheaper but quality alternatives.

In the part of Central European laboratories for 50 years *Shandon Cytospin* centrifuge were used for Non-Gynecological Cytology. The preparations were prepared according to that technology are of good quality. It introduced in the field of cervical cytology tests, but there is lack of jobs, in which would be analyzed the system's effectiveness in screening programs, i.e. in the diagnosis of precancerous cervical lesions. We explore a more recent version of that system - a system of *Shandon PapSpin*. We will analyse the diagnostic efficiency of the system, its benefits. This work will help to assess and use more effective application of cytological centrifugation systems in cervical cytology tests.

1.3 Objective of study

To evaluate the effectiveness of liquid based *Shandon PapSpin* gynecological test in the diagnosis of cervical precancerous pathology.

1.4 Tasks of study

1. Using split-sample cervical material to evaluate the effectiveness of conventional smear and liquid-based *Shandon PapSpin* preparation and to compare their results with appropriate histological tests results.
2. Using direct –to-vial cervical material to evaluate the effectiveness liquid based *Shandon PapSpin* system and to compare the results with appropriate histological tests results.
3. To compare informativeness of direct–to-vial liquid based *Shandon PapSpin* test with informativeness of conventional split- sample cytological test.
4. To evaluate suitability of remaining liquid medium after the cytological test to determine of the HPV by PCR method.

1.5 The scientific novelty of the study

Cytological tests of cells fixed in liquid media using *ThinPrep* and *SurePath* technologies are performed above 13 years. Scientific works proved that these tests allow more effectively to diagnose precancerous cervical lesions than conventional cytological smear test. Studies have shown that results of cytological tests of cervical cells fixed in liquid media will be worse if investigating cytological material is split-sample in comparison with test results of direct-to-vial sample. Therefore implementing of cytology tests systems to clinical practice are guides to evaluate the effectiveness of cytological tests of cervical direct-to-vial sample .

There are a lot of studies of effectiveness of *ThinPrep* and *SurePath* systems, but lack of works about systems whose operating principle on cytocentrifugation basis is felt. There are several systems operating on cytocentrifugation principle: *Shandon PapSpin* (ThermoShandon, Pittsburgh, Pennsylvania, U.S.), Turbitec® (Labonord, Templemars, France) and *CytoSCREEN*® (Seroa, Monaco, Monaco). Our chosen system *Shandon PapSpin*^{TM} is widespread in Western Europe. World literature published several studies analyzing the effectiveness of the system *Shandon PapSpin*^{TM}. Khalbuss at al., Weynand at al., Garbar at al., Rosenthal at al. analyzed the effectiveness of *Shandon PapSpin* system under the determinate cytological cervical lesions.

Shandon PapSpin efficiency of the system has not been evaluated in their work in the following respects, that is sensitivity and specificity of the screening program, effectiveness of the system investigating of all cervical material (be emphasized that they studied the split-sample of cervical cytological material), as well as undeterminate and inappropriate examinations regard of percent variation in. The problem is that the U.S. Food and Drugs Administration, approval requirements for technology applicable in the field of cervical cytological tests, limits assessment of Shandon PapSpin effectiveness, therefore researches of direct cytocentrifugation cytological systems are impossible.

Because of the scientific publications it is not clear what is the effectiveness of the *Shandon PapSpin* system, how reduce percentage diagnosis of undeterminate and uncertain diagnoses in precancerous cervical pathology, as well as the liquid medium remaining after the cytological study suitability for HPV detection by PCR method when tested direct-to-vial, so our made work will provide more information on this system, the application to the cervical screening, and knowledge about opportunities of the PCR reaction performance allow to decide about application of fixed media of these cells for researches.

Since HPV types belonging to the high cancer risk groups are associated with development of cervical cancer, so early diagnosis of sexually transmitted infection and precancerous lesions become relevant and significant. The possibility to determine of human papilloma virus from the remaining liquid medium after cytological test is additional advantage of cytological test systems, in which cervical cells fixed in liquid media are used. Molecular HPV genomic DNA identification methods are some of the most sensitive, of which the most popular and most widely used is the polymerase chain reaction (PCR), and the various HPV-DNA hybridization techniques. Polymerase chain reaction (PCR) is used in scientific researches. In 2005, Garbar at al. performed study suitability of PapSpin^{TM} and Turbitec ® systems were assessed to determine for HPV-DNA by Hybride Capture II hybridization method. However, remains unclear still the suitability of this system for PCR testing, which detection is based on identification of no degenerate HPV DNA.

2. MATERIAL AND METHODS

In 2006-2009 National Centre of Pathology (NCP), Vilnius University Institute of Oncology (VUIO), Vilnius Central, Vilnius Karoliniskes, Vilnius Region Central Outpatients clinics begun study “Evaluation of Effectiveness of Precancerous Cervical Pathology using *Shandon PapSpin* System” received approval of Lithuanian Bioethics Committee No 7 to perform this study. 2945 women were included in to the survey since March 2006 to March 2008.

2.1 Investigative population

Women from 30 to 60 years old, who come for prophylactic check-up to Primary Health Care Centre in accordance with selective health check-up for cervical lesions program approved by the Ministry of Heath Care and who agreed to take part in this study had to sign an agreement form of informed person. From the study was excluded women age: less than 30 and above 60 years old. Determinate pregnancy.

2.2 Cytology testing of conventional and liquid based PapSpin tests

Before the start of the study the training of obstetricians- gynecologists was performed. There were improved their knowledge how to collect and prepare material for cervical cytology.

Women were informed about study, its benefit and possible outcomes. Written consent was signed of each woman included in to the study. For first 1 500 women in split-sample phase double cytological tests was performed. Cytological material was obtain from the surface of the cervix by Rover Wallach Papette brush and conventional cervical smear in accordance with the instruction of smear preparation was prepared. The residual material was used for PapSpin preparations. Broom with residual cytological material was immersed to bottle with fixative PapSpin liquid medium, was rinsed out, after the holder of brush is broke off with care and the brush is remained in the bottle, the bottle is heat-sealed. The bottle was vortex 10 times roundly. The name, surname, birth date of patient, medical institution sending specimen were marked on label on the bottle. The sending form "Sending to test operative and biopsy material No 014/a" was filled too. Cytological smears of women and cervical cytological material of same women in PapSpin liquid medium bottle with filled requisition form No14/a and written consent of woman were send to National Centre of Pathology for investigation. For another cohort of 1450 women only liquid based PapSpin test was performed without conventional test.

The cytological cervical smear was registered in *Pathi*s database and was administered study number for it in National Centre of Pathology. The smear was stained in accordance with modification of Papanicolaou staining technique valid in National center of pathology. After that the microscopic analysis was performed. The criteria listed bellow was evaluated during analysis:

- Background of preparation.
- Microorganisms.
- Cellular composition of smear.
- Visible lesions of cells.
- Presence of transformation zone component.
- Amount of impurities.
- Quality of staining, smear thickness, air-drying artifacts.

There was made conclusion about suitability of smear for cytological test. In accordance with Bethesda system of 2001 was made diagnostic conclusion. The reporting form of test was delayed until report of cytological test of sample fixed in *PapSpin* liquid medium.

PapSpin medium, in which cervical material was fixed, was registered separately from conventional smear. It was registered in register of evaluation of effectiveness of *Shandon PapSpin* test; the study number was administered for sample. This study number was written on the label of bottle of *Shandon PapSpin* liquid medium with cytological material. The number of study registration was written on sandblasted side of PapSpin glass slide, in which cell will be overspread. Marked slide was inserted to special mega funnel place in *Shandon Cytospin* centrifuge so that the marked side of the slide facing to mega funnel tube. The measurement of cell density was performed by cell densitometer *Cytocheck.* If it was found that the cell density was too high to perform cytological test, the content of mega funnel was diluted with fixative solution, see Table 1.

The measurement of specimen cell density by densitometer *CytoCheck***:**

1. *PapSpin* medium is shaken (for at least 10-15 seconds). Wait a few seconds until the disappearance of air bubbles generated at the shake time, and the sample becomes homogeneous.
2. Immediately the container with PapSpin medium fixative cervical cytological material is inserted into the *CytoCheck* device (can not delay too long, because the cells precipitated quickly on the bottom of the container). The sharp edging of brush must be facing to red

zone of the device, because only in that position the brush does not affect the measurement results.

3. Data of evaluation of sample cell density occurs on screen of device.

The filling of mega funnel tube with sample:

1. *PapSpin* container with specimen is gently stirred (the specimen is homogenized) and proper sample volume is taken from container bottom by microdosator in accordance with data of Table 1.

2. The sample is poured to the tube through funnel shaped channel.

3. The proper amount of *PapSpin* fixative solution is added or is not added to tube.

4. Close the tube cap.

Table 1. Dependence of *PapSpin* fixative solution and required to analyze material amount on cell density.

Data of evaluation of specimen cell density in screen of densitometer	Volume of sample which must be added to mega funnel tube	Volume of PapSpine fixative solution which must be added to mega funnel tube
Over cell density	0,2 ml	1,8 ml
High cell density	0,2 ml	1,8 ml
0,2 ml	0,2 ml	1,8 ml
0,3 ml	0,3 ml	1,7 ml
0,4 ml	0,4 ml	1,6 ml
0,5 ml	0,5 ml	1,5 ml
0,6 ml	0,6 ml	1,4 ml
0,7 ml	0,7 ml	1,3 ml
0,8 ml	0,8 ml	1,2 ml
0,9 ml	0,9 ml	1,1 ml
1,0 ml	1,0 ml	1,0 ml
1,1 ml	1,1 ml	0,9 ml
1,2 ml	1,2 ml	0,8 ml
1,3 ml	1,3 ml	0,7 ml
1,4 ml	1,4 ml	0,6 ml
1,5 ml	1,5 ml	0,5 ml
1,6 ml	1,6 ml	0,4 ml
1,7 ml	1,7 ml	0,3 ml
1,8 ml	1,8 ml	0,2 ml
1,9 ml	1,9 ml	0,1 ml
2 ml	2,0 ml	Fixer isn't added
3 ml	2,0 ml	Fixer isn't added
4 ml	2,0 ml	Fixer isn't added
5 ml	2,0 ml	Fixer isn't added
Low cell density	2,0 ml	Fixer isn't added

5. Mega funnel with care (so, that the specimen would not contact with slide) is inserted to cytocentrifuge rotor. The specimen is centrifuged for 5 min, at 1250-rpm.
6. At the end of centrifugation slide with thin-layer smear is removed from mega funnel with care (so, that supernatant would not contact with slide).
7. Slide as soon as possible horizontally placed on a transport pallet
8. The prepared preparation is immediately fixated by it immersing in 96% ethyl alcohol for 10 minutes and is stained by Papanicolaou method in automatic painting apparatus in accordance with the standards validated at the Center "Papanicolaou staining method adapted to the COT 20 MEDITE staining machine".

Prepared preparations were analyzed by microscope. Similar parameters were evaluated during the study as evaluating a simple smear (microorganisms, cellular composition of preparation, impurities, thickness of preparation, fixation, staining quality). There was made conclusion about suitable of preparation to cytological test. In accordance with 2001 Bethesda system of cytological diagnosis evaluating visible by microscope components the diagnostic conclusion was made. Test results were recorded to form of *PapSpin* cytological test data collection form. *PapSpin* and simple smear test results are compared at the end of the working day. As it turned out that results of both tests were the same, the report of simple smear was send to doctor ordered this test. However, if results of *PapSpin* test showed higher grade of lesion than simple smear, so report of simple smear was formulated due to higher grade of pathology.

2.3 Histological examination of cervical material

Obstetrician-gynecologist chose observation tactic of patient according to received cytological test results. For women with cytological ASC-US, LSIL tests results cytological follow-up were repeated and HPV DNA detection was performed. Women with conclusion of ASC-H, HSIL, malignant tumor were send to specify diagnosis and for treatment to VUOI. Performing cervical biopsy or conization in these health institutions histological cervical material fixed in 10% Buffered Formalin Solution was delivered to test to National Centre of Pathology. Histological analysis of cervical conizate was performed in this order:

1. Cervical conizate (cone or fragmented cone) was examined, measured in sampling room.
2. There were described visible cervical mucous and transformation zone lesions. Macroscopic separating procedure of cervical material:
3. Cervical cone is opened along (at12 o'clock), expanded and fixed.
4. There are made longitudinal sections of 0,3 –0,5 cm – at least 3 from each quadrant (at 12-3, 3-6, 6-9, 9-12 o'clock). All material is investigated.
5. Each section is placed in a separate cassette in order. *ThermoShandon* tissue processor in accordance with method valid in National Centre of Pathology fixates the material in cassettes. For microscopic analysis six paraffin sections of 3μm thickness are prepared from 3 levels of specimen. Paraffin sections are stained according to H&E staining technique.
6. All cervical biopsy material is tested. From it is prepared preparations in above described order.

There was performed microscopic histological analysis of preparations. The histological diagnosisn was formulated in accordance with 2003 WHO Classification of Tumors of the Breast and Female Genital Organs and CIN system of 3 grades of cervical intraepithelial neoplasia.

The several parameters was evaluated in histology:

- CIN grade.
- Localization of CIN in excision biopsy.
- Is the lesion multifocal, or not.
- Size of CIN lesion (if it is invasive cervical cancer, then is measured it's horizontal and vertical diameter necessary for determination of cervical cancer stadium).
- Reaction of cervical stroma.
- The status of blood vessels.
- The status of resection margins.
- Other benign cervical findings (tubal metaplasia, microglandular hyperplasia, endometriosis, regenerative and reparative cervical epithelia).
- When determined invasive cervical tumor, it's horizontal and vertical parameter is measured. Stadium of tumor.
- There were described results of performed histochemical and immunohistochemical reactions.

2.4 Detection of HPV DNA in liquid *PapSpin* medium

Total 291 cytological material specimens were tested by PCR method during research in VUIO. Cell obtained from cervical transformation zone were used for Human Papilloma Virus determinate. 2 specimens were collected from patient's cervix during visit to gynecologist. To collect first cytological specimen was used *Wallach Papette* brush and complex of transporter medium: marked *PapSpin* container with fixative *PapSpin* medium. The brush with collected cytological material was immersed to container with *PapSpin* liquid medium, then holder of brush was broke with care, the brush was remained in container. The individual study number was administered for each specimen. The material for test was obtained at not menses time, at 10-20 day of cycle. Before start of analysis the material was stored in special container at room temperature (18 – 20°C. The second cytological specimen was collected to check *PapSpin* medium for HPV test. Sterile brushes and complex of tube with transporter PBS medium was used to collect cell. The brush with cytological material was immersed to PBS medium, rinsed out and broking it's holder was remained in tube. Before testing of HPV the material was stored at -20°C temperature

Detection of HPV using DNA amplification method. Using method of polymerase chain reaction with common primers of human papilloma virus was established is HPV DNA in tested cytological material. Were used MY09/MY11 primers (Table 2).

Table 2. PCR primers MYO9 and MY11 for HPV DNA detection

Primer	Sequence (5'→3')	Amplificated product (bp)
MY09	5'→CGT-CCM-ARR-GGA-WAC-TGA-TC →3'	450 bp
MY11	5'→GCM-CAG-GGW-CAT-AAY-AAT-GG→3'	450 bp

*Where M=C or A, R=G or A, W=T or A, Y=T or C.

Performing PCR, test of material was performed in few stages. Cytological material located in *PapSpin* medium before DNA extraction was washed additionally by PBS buffer solution (pH=7.4), all centrifugations were performing at 20-23ºC temperature:

1. From *PapSpin* medium is taken 2 ml of cells suspension and centrifuged for 10 minutes, at 2000 rpm. (RCF=2880);
2. The supernatant is removed. 500 µl of PBS buffer is poured over on the bottom remaining cells pipetting them easily;
3. Centrifuged for 5 minutes, at 2000 rpm. (RCF=2880);
4. The supernatant is removed. 500 µl of PBS buffer is poured over on the bottom remaining cells pipetting them;
5. Centrifuged for 5 minutes, at 2000 rpm. (RCF=2880);
6. The supernatant is removed and, if need, PBS buffer is added. For DNA extraction is used 200 µl washed cells.

Cytological material immersed directly to tube with PBS buffer additionally wasn't prepared. Remaining brush was removed from tube and residual cytological material was used for follow-up tests.

DNA of part samples (n=162) was extracted using *Genomic DNA Purification Kit,* AB *Fermentas* according to manufacturer recommendations. DNA of other sample (n=129) was extracted by column method using *SorpoClean*^{TM} *Genomic DNA Extraction Module,* SORPO Diagnostics according to manufacturer recommendations.

Protocol of DNA extraction using Genomic DNA Purification Kit, AB *Fermentas*:

1. Mixed of 200 µl cells suspension and 200 µl lysis solution, incubated in water bath for 10 minutes at 65° C temperature.

2. Added of 600 µl chloroform, gently shaken until homogenous emulsion, centrifuged for 5 minutes in microcentrifuge at 14 000 rpm (RCF=20160).
3. From 10-times precipitation solution is prepared working solution mixing 80 µl 10-times precipitation solution with 720 µl of sterile deionized water.
4. Upper part of aqueous centrifugate carefully is transferred to prepared precipitation solution, gently mixed, a few minutes left at room temperature, then centrifuged for 5 minutes at 14 000 rpm. (RCF=20160).
5. The supernatant is removed, pumped out all remainders and residual DNA is thawed gently shaking of 100 µl 1,2M NaCl solution. Visually checked did DNA thaw completely.
6. Added 300 µl of a cold ethanol (96%), DNA is incubated for 20 hours at -20°C temperature. Taken after incubation samples are centrifuged for 5 minutes at 14 000 rpm (RCF=20160). The supernatant is removed. Precipitate is washed with 75% ethanol and thawed gently shaking in 100 µl sterile deionized water. Visually checked did DNA thaw completely.

Protocol of DNA extraction using the column method (SORPO Diagnostics):

1. **Cells lysis (destroying):**
    - Samples must be at room temperature (15 – 20°C);
    - Added of 20 µl proteinase K to a 1.5 ml microcentrifuge tube;
    - Added of 200 µl sample;
    - Added of 200 µl lysis solution;
    - Mixed gradually shaking for 10 seconds until a homogeneous solution;
    - Centrifuged for 2 seconds shortly that would remove drops from inner of tube cap;
    - Samples are incubated for 10 minutes in 56°C water bath shaking every 2 minutes.
2. **Transfer of material on the column:**
    - Removed samples from water bath added at a 230 µ of ethanol (96-100%) to each sample;
    - Samples mixed gradually shaking for 10 seconds;
    - The mix carefully is transferred from each tube to prepared columns (they are in 2 ml collection tubes);
    - The cup of column is closed and samples are centrifuged at 6000 x g for 1 minute;
    - The lower tube with filtrate is ejected; the column is transferred to clean 2 ml collection tube. If after centrifugation the lysate filtrated through column incompletely, it is centrifuged one more time at a higher rate.
3. **Wash:**
    - The column is opened and added 0.5 ml diluted **washing buffer solution 1**;
    - The lid is closed and samples are centrifuged at 6000 x g for 1 minute;
    - The lower tube with filtrate is ejected; the column is placed to clean 2 ml collection tube;
    - The lid of column is opened and added 0.5 ml diluted **washing buffer solution 2**;
    - The lid of column is closed and centrifuged at maximum speed (20000 x g) for 3 minutes;
    - The lower tube with filtrate is ejected; the column is placed to early-prepared 1.5 ml collection tube.

4. **DNA wash:**
    - The column is opened and added 100 µl of until 70°C heated **washing buffer solution**;
    - The lid of column is closed and samples are incubated for 2 minutes at room temperature;

- After incubation centrifugating for 1 minute at 6000 x g;
- Washing procedure repeated one more time not exchanging lower collection tube;
- For PCR is used of 5 – 10 µl extracted DNA depending on obtained DNA concentration.

The measurement of DNA concentration by biophotometer.

The concentration of extracted DNA was measured by BioPhotometer, *Ependorff* in VU Department of Genetics, Faculty of Natural Sciences. Process of work:

1. The samples gradually are shaken, the drops are ejected from walls during centrifugation;
2. Preparing 50 µl of 10-times dilute sample (diluting with distilled water) Nature of dilution may be chosen depending on how much obtaining of extracted DNA and that would remain enough for further exploration;
3. Prepared samples are vortexed, drops from walls are centrifuged;
4. All volume of tube is transferred to cuvette (*Ependorff*).
5. Datum points of measurement are estimated before placing cuvette to biophotometer to measure concentration of sample. This is done by adding the same amount of distilled water to another cuvette and placing it to biophotometer;
6. DNA concentration is measuring. The resulting values are recorded;
7. For next sample is using the same cuvette, before rinsing it several times with distilled water.

In next stage the amplification of extracted DNA was performed by PCR method. For HPV detection common primers MY09 and MY11 were used (Table 4), also *REDTaq® ReadyMix™ PCR Reaction Mix with $MgCl_2$* (SIGMA, USA). Composition of PCR solution: 20 mM Tris-HCl (pH 8.3), 100 mM KCl, 3 mM $MgCl_2$, 0.002 % gelatin, 0,4 mM dNTP mix (dATP, dCTP, dGTP, dTTP), stabilizers, 0,06 U/µl Taq DNA polymerase**.** PCR was performed using thermocycler (BIO-RAD, Germany), and program was chosen according to chosen primers.

DNA amplification at PCR reaction

1. There was prepared mix for PCR reaction. Quantities of all materials of common reaction mix are calculated depending on samples number. To each sample added of 10 – 15 µl mix:
   - 10–11µl REDTaq® ReadyMix™ reaction with $MgCl_2$;
   - 1–2 µl of primer MY09 and primer MY11;
2. There PCR reaction mix is added to tubes and 5–10 µl of testing DNA depending on concentration of testing DNA. The reaction is performed at 25 µl volumes.
3. Prepared tubes are placed to PCR thermocycler and required program is set up. Our PCR program lasted for 3 hours 30 minutes:
   - At first one primary denaturation cycle was performed at 93° C temperature, its duration 3 minutes.
   - Followed 40 cycles each consisting of:
     - Denaturation for 1 minute at 94°C temperature,
     - Annealing of the primer for 1 minute at 55°C temperature,
     - Elongation of the primer for 1 minute 30 seconds at 72°C temperature.
   - At the end is performed extension of the primer 1 cycle at 72°C temperature that lasted for 5 minutes

PCR amplification products are analyzed using electrophoresis. Electrophoresis is performed in 2% agarose gel stained by ethidium bromide. For electrophoresis was taken 6 µl of PCR amplification product and was loaded into agarose well without additional stain to observe PCR product because to manufacturer PCR reaction mix stain has been added. After electrophoresis stained by ethidium bromide products were analyzed in transilluminator (HEROLAB, Belgium and Germany). The results were photographed.

2.5 Statistical methods

Quantitative variables are presented as mean and standard deviation; categorical variables are presented in absolute and percentage term. There was evaluated percentage correlation between cervical diagnostic tests using of a match analysis. The correlation between simple smear test and tests of *PapSpin* liquid medium preparations detecting cervical precancerous lesions was evaluated using Kappa measure of agreement (κ). Kappa was calculated using dichotomous classification (positive and negative) of pathological lesions. When values of Kappa measure was 0,81-1, the agreement, was interpreted as excellent, 0,61-0,8 - good, 0,41-0,6 – moderate, 0,21-0,4 – marginal (fair), less than 0,2 – poor.
The sensitivity and specifity of tests stage of split-sample and direct-to-vial of cytological tests were calculated on the basis of correlation tables of histological and cytological tests methods. When histologically was confirmed pathology of CIN2/CIN3/SCC and malignant tumor, then cytological diagnosis was evaluated as correct positive HSIL detecting. To evaluate differences between groups χ^2 was used. Confidence intervals were calculated. The difference was significant when *p* was at confidence interval or *p* value was less then 0,05. ROC curves were used to determine diagnostic test accuracy evaluating the area under the curve. Diagnostic test value was interpreted as excellent when the area under curve was 0,9-1, as very good - 0,8-0,9, as good - 0,7-0,8, as satisfactory – 0,6-0,7, as unsatisfactory - 0,5-0,6.
To evaluate postoperative residual disease, lesion of resection margin status *Odds ratio* was calculated and its 95 % confidence interval. The risk of postoperative residual disease depending on resection margin status was evaluated by Odss ratio. Statistic analysis was performed using „Microsoft Excel“ (*Microsoft Corp., Redmond, WA, USA*) and SPSS 13 (*SPSS Inc., Chicago, Ill, USA*).

3. Results

A total of 2,945 women were examined during the study. All the women were grouped into six age groups with five year intervals: 30–34 in group I, 35–39 in group II, 40–44 in group III, 45–49 in group IV, 50–54 in group V, and 55–60 in group VI (Table 3). The mean age of women was 42.4 years, the youngest was 30 and the oldest was 60 years old (SD ± 9.0). The youngest women (Group I) accounted for 25.9% of the samples and the oldest women (Group VI) for the only 10.5%. There were no differences in age distribution between study group .

Table 3. Number of women according to age groups participated in the screening study.

Age group	Age	Number of women	%
I	30-34	762	25,9
II	35-39	485	16,5
III	40-44	441	15
IV	45-49	533	18,1
V	50-54	415	14,1
VI	55-60	309	10,5

4945 cervical tests were performed during the study. 189 (6.4 %) abnormal cytology cases were found. Double cytological analysis of 1500 women was performed in study stage of split-sample: 1500 cervical cytological smears and 1500 *PapSpin* preparations. Results of double cytological test allowed detecting cervical pathology for 104 (6,8%) women. Precancerous CIN2/CIN3/CIS cervical lesions were detected and treated mostly to women in 30 – 40 years age group. CIN1 pathology was diagnosed more frequent to women of 30-34 years old.

3.1 Split-sample phase results

Analyzing conventional cervical smears and *PapSpin* preparations according to adequacy there was determined statistically significant difference between two methods. With conventional smear there were found 25 (1,7%) unsatisfactory for cytological evaluation cases comparing with 6 (0,4%) PapSpin cases (p=0,001).

Different percent of pathological cases was detected by cervical conventional smear and *PapSpin* methods. According conventional smear was detected 6% abnormal cytology cases, *PapSpin* -5,3%. Results of split-sample study tests is presented in table 4.

Table 4. Cytological results of split-sample study phase.

	Split sample phase (n=1500)	
	Smear	*PapSpin*
Bethesda category	(%)	(%)
NILM	1387 (92, 5)	1416 (94,5)
Unsatisfactory	25 (1,7)	6 (0,4)
ASC-US	31 (2,1)	10 (0,7)
ASC-H	4 (0,3)	3 (0,2)
LSIL	20 (1,3)	29 (1,9)
HSIL	28 (1,9)	33 (2,2)
AGC	3 (0,2)	1 (0,1)
AIS	1 (0,1)	-
Squamous Cell Carcinoma	1 (0,1)	1 (0,1)
Metastasis of stomach carcinoma	-	1 (0,1)
Total	1500	1500

Higher number of ASC–US diagnosis was detected by conventional smear method – 31 cases (2.1 %) for comparison than *PapSpin* method - 10 cases (0.7 %) (p=0.001) in study stage of split-sample. The result is statistically significant. Reasons of higher number of ASC-US diagnosis was mechanically destroyed cells preparing simple smear, air-drying artifacts with delay fixation, scant diagnostic material.

The higher number of LSIL cases was detected analyzing cervical cells by *PapSpin* method – 29 cases (1,9%), in comparison with 20 cases (1,3%), detected by simple smear method, however there wasn't a statistically significant difference in diagnosing of this pathology (p=0.12). Analyzing cells of simple smear and by *PapSpin* method prepared to microscopic analysis, was revealed that optimal cells fixation that allows to see the light around cells nuclei and nuclear atypia due to cells lesion – koilocytes caused by HPV infection, in *PapSpin* preparations led to a better diagnosis of LSIL cases. It is shown LSIL cells visible analyzing simple smear in Figure 33, LSIL cells visible in *PapSpin* preparation. There was established lower number of cases of HSIL and malignant tumors in study stage of split-sample by simple smear method 28 (1.9%) than by *PapSpin* method -33 (2.2%), however this difference isn't statistically significant (p=0,33).

Coincidence of tests results of cervical cytological smears and *PapSpin* preparations is shown in Table 6. 95% of test results were concordant.

Table 5. The agreement of conventional smear and *PapSpin* preparations in split-sample phase.

	Conventional smear									
PapSpin	Unsatisfactory	NILM	ASC-US	ASC-H	LSIL	HSIL	AGC	AIS	SCC	*Total PapSpin*
Unsatisfactory	6	-	-	-	-	-	-	-	-	6
NILM	18	1371	20	1	1	2	2	1	-	1416
ASC-US	1	7	2	-	-	-	-	-	-	10
ASC-H	-	1	1	1	-	-	-	-	-	3
LSIL	-	4	7	-	18	-	-	-	-	29
HSIL		2	1	2	1	26	1	-	-	33
AGC	-	1	-	-	-	-	-	-	-	1
Metastatic Carcinoma of stomach	-	1	-	-	-	-	-	-	-	1
Squamous Cell Carcinoma	-	-	-	-	-	-	-	-	1	1
Total smears	25	1387	31	4	20	28	3	1	1	1500

Kappa correlation coefficient 0,87 (95% CI:0.81-0.92) shows excellent coincidence of results of both methods. The correlation of cytological and histological results in split-sample phase is presented in table 9. The split-sample *PapSpin* test sensitivity were 68.7% *vs* 78.1%, the specifity - 93.8% *vs* 91.8% respectively. Positive prognostic value of conventional smear and *PapSpin* was statistically significantly indifference - 88,0% and 86,2%. There wasn't estimated statistically significant difference of negative prognostic value of analysis too - 82,1 *vs* 86,8 %.

Table 7. Correlation of cytological and histological results in split-sample phase

	Conventional smear						
Histological diagnosis	WNL	ASC-US	ASC-H	LSIL	HSIL	AIS	SCC
Benign lesions	26	5	1	2	1		
CIN1		4		8	2		
CIN2				2			
CIN3/CIS	1	2	3		20	1	
Malignant Tumor	2						1
	PapSpin™						
	WNL	ASC-US	ASC-H	LSIL	HSIL	Carcinoma	SCC
Benign lesions	29	2		3	1		

CIN1	2	1		8	3			
CIN2				2				
CIN3/CIS	2		2		23			
Malignant Tumor			1			1	1	

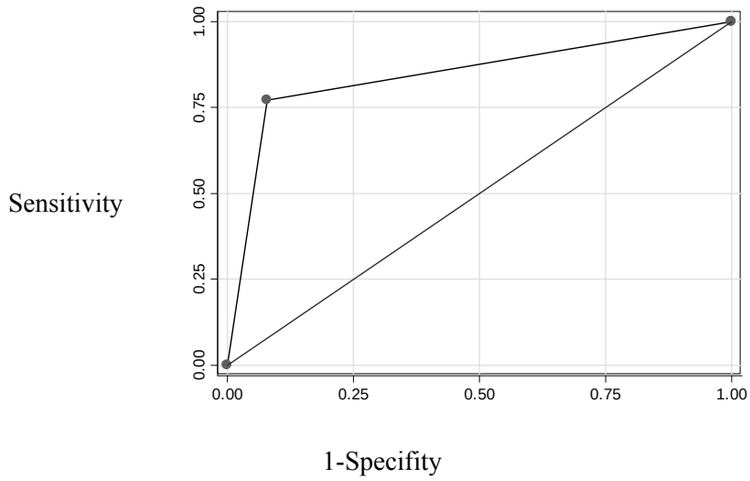

Figure 1. ROC curve of split sample phase conventional smear.

The area under the curve is 0,8469 (95% CI: 0,78-0,91), that shows very good accuracy of test (Figure 1).

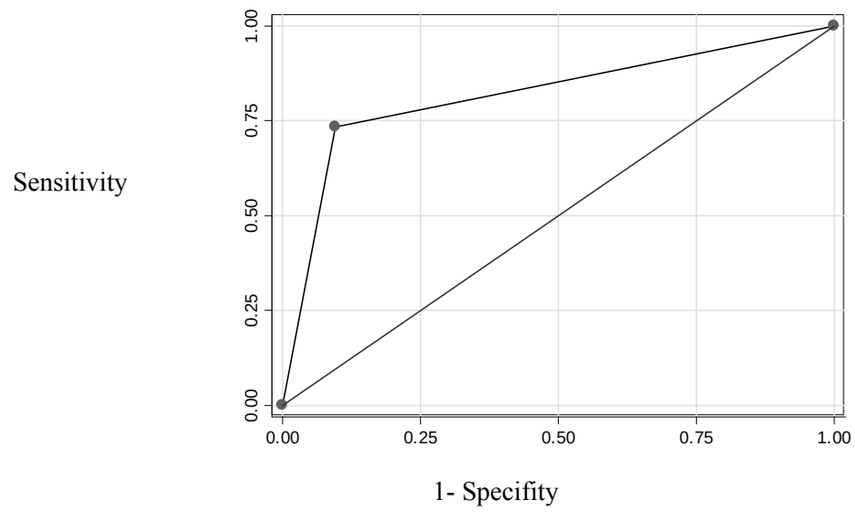

Figure 2. ROC curve of split- sample liquid based PapSpin test .

The area under the curve is 0,8190 (95%PI:0,72-0,91), that shows very good accuracy of test (Figure 2).

Preparations prepared by *PapSpin* method comparing with conventional smears are of optimal fixation, good quality that are controlling in the laboratory. Homogenous cells suspension is equally overspread on area of slide. There aren't impurities – blood, inflammatory exudate. Morphological signs of dysplastic cells and tissue fragments are identified with all diagnostic criteria. Microscopic analysis of *PapSpin* preparation is less tyring than conventional smear.

3.2 Direct-to-vial phase results

Analyses of 1450 *PapSpin* preparations were performed. All material was examined by *PapSpin* method. Cytological analysis of preparations obtained by *PapSpin* method

allowed detecting 85 (5,9 %) of abnormal cytology cases. Direct-to-vial phase cytological results is presented in table 8.

Table 8. Direct-to-vial phase liquid based cytology results..

	II study stage (n=1445)
Bethesda category	*PapSpin,* %
WNL	1352 (94,5)
Unsatisfactory	8 (0,6)
ASC-US	20 (1,4)
ASC-H	10 (0,7)
LSIL	22 (1,5)
HSIL	31 (2,1)
AGC	2 (0,1)
AIS	-
Squamous Cell Carcinoma	-
Total	1445

There were detected 31 (2.1%) of HSIL+ and 22 (1.5%) of LSIL cases in study stage of direct-to-vial sample comparing with 28 (1,9) *vs* 20 (1,3) of study stage of split-sample. Low percent of ASC-US and ASC-H cases - 1,4% and 0,7% - essentially the same as analyzing cytological material in study stage of split-sample by *PapSpin* method – 0,7% and 0,2% respectively. Percent of unsatisfactory preparations was the same as in study stage of direct-to-vial sample - 0,6% and 0,4% respectively. This percent is three times less than of unsatisfactory conventional smears in study stage of split-sample.

There were performed 60 conisations in study stage of direct-to-vial sample. Comparison of cervical cytological and histological analyses of studying women is shown in Table 9.

Table 9. Comparison of liquid based *PapSpin* preparation and histology in direct-to-vial phase.

	PapSpin						
Histological diagnosis	WNL	ASC-US	ASC-H	LSIL	HSIL	Carcinoma	Unsatisfactory
Benign lesions	15	1	2	5	1		1
CIN1			1	4			
CIN2		1	1		5		
CIN3/CIS		1	3	1	16		
Carcinoma					1		1

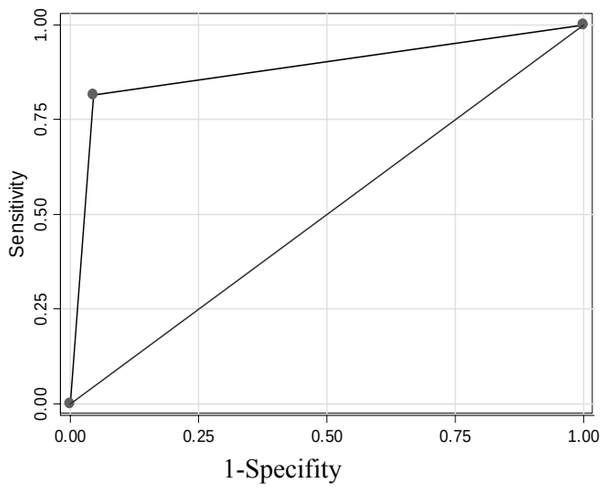

Figure 3. ROC curve of liquid based *PapSpin* preparation in direct-to-vial phase.

The area under the curve is 0,8847 (95% PI:0,79-0,97), that shows high accuracy of test (Figure 3).

The sensitivity of *PapSpin* analysis – 75.9%, specifity –96.5% Positive prognostic value of test– 95,7%, negative prognostic value of test 80,0%. There weren't detected statistically significant differences between sensitivity, specifity, positive and negative prognostic values of analyses of first study stage of conventional smear and *PapSpin* preparation ($p<0,05$).

3.3 Results of histological analysis

Analyzing histological cervical material was revealed additional information. 141 surgical operations were performed at: 57 conizations, 68 biopsies, and 19 hysterectomies. Indications for surgery were: cervical tests showed pathology (94), cervical endometriosis (1), uterine leiomioma (6), adenocarcinoma of the endometrium (3), ovarian serous cystadenoma (1), dysgerminoma (1), ovarian adenocarcinoma (2), pathological colposcopic findings (15), any information (17).

There was performed cervical conization for 58 women participated in study due to CIN3/CIS. Performing histological analysis in 26 (44,8 %) cases was established CIN3/CIS in conization section margins, in 20 (34,5%) cases was detected that CIN3/CIS totally excised, in 12 (20,7%) cases the affection of resection margins wasn't noted. By macroscopic nature of material obtained to test fragmentized cervical material dominated – 33 (59%) cases, in 24 cases (41 %) was obtained solid cone.

In followed study we used information received from database of National Centre of Pathology about observation after surgery of 48 (84%) women from operated 58. This observation consisted from cytological tests of smears and repeat conizations. From 48 observed women 11 (44 %) of women, in which resection margins were visible CIN3/CIS (Figures 45, 46), there was performed postoperative cytological test at it was detected HSIL, wherefore were repeated conization for these women. Declared in histological test conclusions CIN3/CIS spreading in resection margins of 14 (56%) women weren't confirmed performing latest cytological analyses (10 cases) or repeat conization (4 cases). Higher risk according to results of cytological analysis after conization was associated with resection margin status (Table10). During observation of disease spreading the risk was 20 times higher at a defined dysplasia in resection margins (OR=20,0).

Radicality	Observation: Residual disease detected	Observation: Residual disease undetected	Odds ratio (95 % CI*)
Affected resection margins	11	14	20, 0 (2,0 - 159,3)
Unaffected resection margins	1	18	1,0 (ref.)

Table 10. The relation of residual cytological HSIL and resection margin status .

* CI – confidence interval

Resection margins of 25 women were affected CIN3/CIS. Endocervical and deep resection margins of 7 women were affected, of 18 – endocervical resection margin. For 5 (34%) women with affected endocervical resection margins HSIL was detected by postoperative cytological test. HSIL was detected by cytological test for 7 (85%) women with affected endocervical and deep resection margins, after 4 months after surgery. There was established signally higher risk of recidive when CIN3/CIS affections are in two resection margins (OR=161,0) than in one (OR=10,5).

Table 11. HSIL detected performing cytological analysis during observation dependence on CIN3/CIS in one and two resection margins

Resection Margins Status	Observation: Determined residual disease	Observation: Undetermined residual disease	Odds ratio (95 % CI*)
Affected endocervical and deep margin	6	1	161,0 (5,9-2006,1)
	5	13	10,5 (0,9-88,2)
Affected endocervical margin Any affection	1	18	1,0 (ref.)

* CI – confindence interval

3.4 HPV DNA detection

PapSpin vial with cervical material after cytological testing were send for HPV detection. As *PapSpin* medium control of virusology test were send and fresh (new) specimen in PBS medium corresponding to No of PapSpin medium. 176 samples were examined due to human papilloma virus in total: 160 *PapSpin* media and 160 samples newly collected of unfixed material. For 16 women samples *PapSpin* medium corresponding newly collected unfixed material wasn't taken.

Comparison of DNA concentrations of study groups. DNA was extracted using Genomic *DNA Purification Kit,* AB *Fermentas* according to manufacturer recommendations. And using genomic DNA extraction kit (*SorpoClean*^{TM} *Genomic DNA Extraction Module,* SORPO Diagnostics) according to manufacturer recommendations.

	DNR concentration, averages((µg/ml)	DNR concentration, min (µg/ml)	DNR concentration, max (µg/ml)	±SD
PapSpin vial (n =160)	11,1	1,0	58,0	20,1
Control sample fresh material (n=16)	67,5	2,0	201,0	45,5

Table 12. Averages of DNA concentrations using different DNA extractions methods

There was a statistically significant difference between the study and controls samples concentrations (11.1 µg/ml and 67.5 µg/ ml, respectively, $p < 0.0001$) (Table 12). The average concentration of extracted DNA from fresh material (control samples) was higher than the average concentration of DNA from PapSpin liquid (case samples). According to these data we assume that using the same DNA extraction technique according to the manufacturer protocol, the cells are lysated and DNA is extracted better from fresh material than from the material with preserving agents (liquid PapSpin medium).

HPV DNA detection in study groups. HPV DNA was detected analyzing extracted DNA. By PCR method was amplified DNA, and amplified products, performing electrophoresis in 2% agarose gel stained with ethidium bromide, were analyzed in transilluminator using UV light. DNA product of human papilloma virus is of 450 base pairs length.

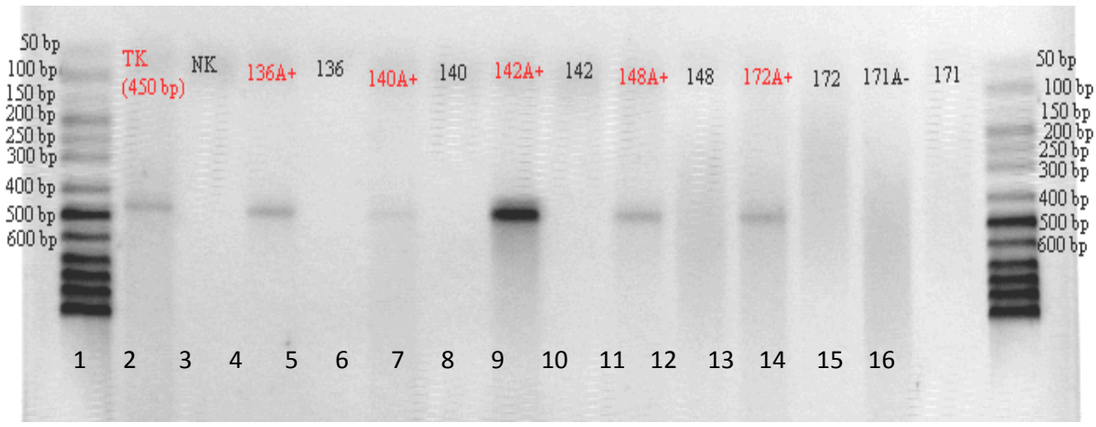

Figure 4. Analysis of electrophoresis PCR products of study and control groups specimens

1, 16 lanes – standard of 50bp DNA length

2 lane – positive HPV control

3 lane – negative HPV control

4 lane – 136A+ - specimen of HPV positive control group

5 lane – 136 – specimen of HPV negative study group

6 lane – 140A+ - specimen of HPV positive control group

7 lane – 140 – specimen of HPV negative study group

8 lane – 142A+ - specimen of HPV positive control group

9 lane – 142 – specimen of HPV negative study group

10 lane – 148A+ - specimen of HPV positive control group

11 lane – 148 – specimen of HPV negative study group

12 lane – 172A+ - specimen of HPV positive control group

13 lane – 172 - specimen of HPV negative study group

14 lane – 171A- specimen of HPV negative control group

15 lane – 171 – specimen of HPV negative study group

In figure 48 samples of study group 136, 140, 142, 148, 172 and 171 are HPV negative (signed with black color). Respectively samples of control group (signed with red color) 136A, 140A, 142A, 148A, 172A are HPV positive, and sample 171A of control group – negative (signed with black color). As positive PCR control CaSki cells were used (cervical cells infected with 16 HPV). The distillated water was used as negative control.

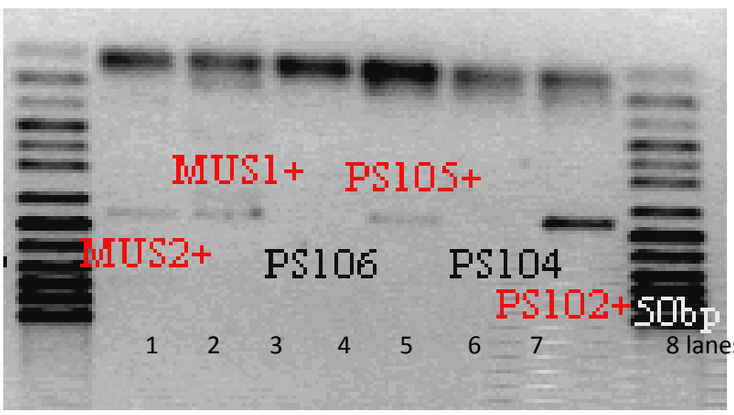

Figure 5. Analysis of electrophoresis PCR products of study and control groups specimens

1,8 lanes – standard of 50bp DNA length

2 lane – MUS2+ - specimen of HPV positive control group

3 lane – MUS1+ - specimen of HPV positive control group

4 lane – PS106 - specimen of HPV negative study group

5 lane – PS105+ - specimen of HPV positive study group

6 lane – PS104 - specimen of HPV negative study group

7 lane – PS 102+ - specimen of HPV positive study group

HPV positive samples are imaged with red color in Figure 25. MUS1 and MUS2 are samples of control group, both HPV positive. PS106 and PS104 – samples of study group, both HPV negative. Samples of study group PS105 and PS102 are HPV positive. To measure of PCR products was used standard of 50bp DNA length. Results of tests were photographed and saved. Analyzed all samples results are summarized and shown in table 13.

Table 13. Comparison of HPV results of study and control groups

	PapSpin vial (n=160)		Fresh material (n=16)		
	n	%	n	%	p
ŽPV positive	9	5,6	8	50,0	p<0,000001
ŽPV negative	151	94,4	8	50.0	

Using PCR the DNA was amplified, and the PCR products, after electrophoresis in 2% agarosis gel stained by ethidium bromide, were analysed in transiluminator using UV light. There was a statistically significant difference between the infection with HPV of study and control samples: there were only 5.6% (9/160) HPV positive study samples, whereas the number of HPV positive control samples was as high as 50.0% (8/16) ($p < 0.000001$) (Table 13). It is interesting to note, that the HPV positive control samples were HPV negative in PapSpin medium. 3 of HPV positive control samples were diagnosed as LSIL, 3 as HSIL and 2 as ASC-H. These data allow us to presume that fresh material is more suitable for HPV DNA extraction and detection than the fixating PapSpin liquid material: HPV DNA was detected 8.9 times more frequently in random controls that all controls in study samples.

4. Conclusions

1. Comprising diagnostic value of cytological tests of preparations prepared by *Shandon PapSpin* system and simple smear in study stage of split-sample was revealed that cytological tests of *PapSpin* preparations are not less accurate than tests of simple smear, although system of liquid media was influenced by material split in this study stage. Sensitivity of *Shandon PapSpin* - 78,1%, of simple smear - 68,7%, specifity 91,8% *vs* 93,8 % respectively. Although determined differences were statistically insignificant ($p< 0,05$), better trend of effectiveness were observed analyzing of *Shandon PapSpin* preparations. There was established statistically significantly less quantity of ASC-US conclusions analyzing *Shandon PapSpin* preparations then analyzing simple smears: 10(0,7%) *vs* 31(2,1%) (p=0.001). There was determined statistically significantly less quantity of preparations unsuitable for cytological test in cytological cervical preparations prepared by *Shandon PapSpin* method then quantity of simple smears: 25(1,7)% *vs* 6(0,4)% (p=0,001).

2. Direct-to-vial *Shandon PapSpin* liquid based gynecological test can be accurately to determine precancerous cervical lesions by cytological test of *Shandon PapSpin* liquid based preparations. Sensitivity of liquid based test - 75,9%, specifity - 96,5%, positive prognostic value of test - 95,7%, negative prognostic value of test-80, 0%.

3. Direct-to-vial phase *PapSpin* gynecologycal test is more effective for comparison to conventional smear according lower unsatisfactory ratio 8 (0,6%) and 25 (1,7%) , ASCUS ratio 20 atv.(1,4%) ir 31 atv.(2,1%) .

4. Liquid *PapSpin* medium is less acceptable for HPV DNA detection by PCR method using MYO9/MY11 primers then cervical material without fixation.

LIST OF PUBLICATIONS ON THE TOPIC OF THE DISSERTATION

1. Gudlevičienė Ž., Juškevičienė G., Rimienė J., Kurlianskienė E., Didžiapetrienė J., Drulia E. Detection and typing of human Papilloma viruses in cervical scrapes of women with cervical carcinomas, squamous intraepithelial lesions and of healthy women. Acta medica Lithuanica. 2002. Supp 19. 40-43.
2. S.Uleckienė, D.Kanopienė, Ž.Gudlevičienė, E.Drulia, J.Kurtinaitis, J.Rimienė, D.Vaitkienė. Atrankinė moterų patikra dėl gimdos kaklelio patologijos.// Internistas. 2004, Nr.6(36) psl. 94-99, Nr.7(37) psl. 94-99.
3. Vaitkiene D., Kurtinaitis J., Armonaviciene A., Kulik J., Uleckiene S., Kanopiene D., Rimiene J. Atrankinės patikros del gimdos kaklelio vėžio eiga Lietuvoje // Lietuvos akušerija ir ginekologija. 2005. Birželis.
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RESUME IN LITHUANIAN

Gimdos kaklelio ikinavikinės patologijos įvertinimo efektyvumas naudojant Shandon PapSpin sistemą

ĮVADAS

Sergamumo gimdos kaklelio piktybiniais navikais mažinimas – prioritetinė sveikatos apsaugos sritis. Lietuvoje sergamumas gimdos kaklelio vėžiu didžiausias Baltijos šalių regione ir antroje vietoje Rytų Europoje po Rumunijos. 2003 m. gruodžio 3 d. LR Vyriausybė patvirtino Valstybinę vėžio profilaktikos ir kontrolės 2003-2010 metų programą, kurios pagrindinis tikslas - sumažinti sergamumą piktybiniais navikais, mirtingumą nuo šių ligų, sergančiųjų onkologinėmis ligomis invalidumą. Tikėtasi, kad atliekant atrankinę gimdos kaklelio patologijos patikrą 30 proc. sumažės susirgimų gimdos kaklelio vėžiu skaičius. 2004 m. liepos mėn. Lietuvoje buvo pradėta Atrankinės patikros dėl gimdos kaklelio patologijos programa. Vykdant atrankinę patikrą naudojamas citologinis gimdos kaklelio tepinėlio testas. Didelė dalis patikros programos efektyvumo priklauso nuo patikroje naudojamo tyrimo kokybės. Pastebėta, kad gerinant patikroje naudojamo testo efektyvumą pasireiškia netiesioginė įtaka sergamumo ir mirtingumo mažėjimui.

Reikia pažymėti, kad citologinio gimdos kaklelio tepinėlio tyrimo efektyvumas nebuvo vertintas prospektyviniuose tyrimuose, tačiau buvo įrodytos šio tyrimo galimybės ir privalumai diagnozuojant ankstyvuosius gimdos kaklelio ikivėžinius pakitimus. Citologinio tepinėlio tyrimas nėra tobulas, jam būdingi klaidingai neigiami ir klaidingai teigiami tyrimo rezultatai. Citologinio tepinėlio tyrimo jautrumo vidurkis - 49%, specifiškumo vidurkis - 95% . Taip pat tiriant šiuo būdu gimdos kaklelio ląsteles yra didelis netinkamų, t.y. nekokybiškų tyrimų skaičius, didelis neapibrėžtų kategorijų procentas. Taip pat dažnai citologinis tepinėlis yra storas, su gausiomis priemaišomis, ryškiais fiksacijos artefaktais, deformuotomis ląstelėmis. Analogiški tepinėliui būdingi trūkumai nustatomi ir Lietuvoje vykdant gimdos kaklelio patikros programą. VLK „Sveidros“ informacinės sistemos duomenimis, tiriant tepinėlius konstatuojamas didelis 5-12% netinkamų tepinėlių kiekis, didelis neapibrėžtų ASC/ASC-H diagnozių procentas, nepakankamai aptinkama HSIL atvejų. Viena pagrindinių priežasčių - nepakankamos kokybės citologinis tepinėlis, sąlygotas tepinėlio ruošimo metodikos trūkumų. Sakysim, įrodyta, kad 50% ląstelių nuo medžiagos paėmimo instrumento ruošiant tepinėlį nepakliūna ant objektinio stiklelio ir yra išmetama su instrumentu.

JAV nuo 1990 m. gimdos kaklelio ląstelių citologiniai tyrimai atliekami naudojant skystosiose terpėse fiksuotas gimdos kaklelio ląsteles. Pasaulinėje literatūroje gausu mokslinių publikacijų, kuriose analizuojamas *ThinPrep* ir *SurePath* sistemų diagnostinis tikslumas, taip pat įrodytas jų poveikis patikros programų efektyvumui. Naudojant *ThinPrep* ir *SurePath* sistemas pasaulyje atliekama 70% gimdos kaklelio citologinių tyrimų. Šioms dviem sistemoms monopolizuojant gimdos kaklelio tyrimų sritį skystosiose terpėse fiksuotų ląstelių citologiniai tyrimai tapo brangūs. Kaštų-naudos analizė pradėjo versti ieškoti pigesnių, tačiau kokybiškų alternatyvų.

Dalyje Centrinės Europos laboratorijų jau 50 metų neginekologinės citologijos tyrimams buvo naudojama *Shandon Cytospin* citologinė centrifuga. Pagal šią technologiją paruošti preparatai pasižymi gera kokybe. Ją pradėta taikyti gimdos kaklelio citologinių tyrimų srityje, tačiau trūksta darbų, kuriuose būtų analizuojamas šios sistemos efektyvumas vykdant atrankines patikros programas, t.y. diagnozuojant ikivėžinius gimdos kaklelio pakitimus. Tyrinėsime minėtos sistemos naujesnį variantą – *Shandon PapSpin* sistemą. Mūsų atiktas darbas leis įvertinti šios sistemos diagnostinį efektyvumą, jos privalumus. Šis darbas leis efektyviau pritaikyti citologinio centrifugavimo sistemas atliekant gimdos kaklelio citologinius tyrimus.

Darbo tikslas

Įvertinti skystojoje terpėje fiksuotų gimdos kaklelio ląstelių citologinio tyrimo naudojant Shandon PapSpin™ sistemą efektyvumą diagnozuojant gimdos kaklelio ikinavikinę patologiją.

Darbo uždaviniai

1. Naudojant dalytą* (*angl*. split-sample) gimdos kaklelio medžiagą, išanalizuoti gimdos kaklelio tepinėlio citologinio tyrimo (Pap testo) ir skystojoje terpėje fiksuotų gimdos kaklelio ląstelių citologinio tyrimo naudojant Shandon Papspin™ sistemą informatyvumą bei palyginti jų rezultatus su atitinkamais histologinio tyrimo rezultatais .
2. Naudojant nedalytą* (*angl*. direct –to-vial) gimdos kaklelio medžiagą išanalizuoti ir palyginti skystojoje terpėje fiksuotų gimdos kaklelio ląstelių citologinio tyrimo naudojant *Shandon Papspin*™ sistemą rezultatus su atitinkamais histologinio tyrimo rezutatais.
3. Palyginti nedalytos gimdos kaklelio medžiagos citologinio tyrimo naudojant Shandon Papspin™ sistemą informatyvumą su gimdos kaklelio tepinėlio citologinio tyrimo informatyvumu.
4. Įvertinti likusios po citologinio tyrimo skystosios terpės tinkamumą nustatyti ŽPV PGR metodu.

Darbo naujumas

Skystosiose terpėse fiksuotų ląstelių citologiniai tyrimai naudojant ThinPrep™ ir SurePath™ technologijas atliekami jau daugiau nei 13 metų. Moksliniais darbais įrodyta, kad šie tyrimai leidžia efektyviau diagnozuoti ikivėžinius gimdos kaklelio pakitimus nei įprasto citologinio tepinėlio tyrimas. Tyrimais įrodyta, kad skystosiose terpėse fiksuotų gimdos kaklelio ląstelių citologinių tyrimų rezultatai bus prastesni, jei tiriama citologinė medžiaga dalyta,, palyginti su nedalytos medžiagos tyrimų rezultatais. Todėl diegiant citologinio tyrimo sistemas į klinikinę praktiką vadovaujamasi nedalytos gimdos kaklelio medžiagos citologinio tyrimo efektyvumo įvertinimo tyrimais .

ThinPrep ir SurePath sistemų efektyvumo tyrimų gausu, o darbų apie sistemas, kurių veikimo principas paremtas citocentrifugavimu, trūksta. Citocentrifugavimo principu veikiančių sistemų yra keletas: *Shandon PapSpin*™ (ThermoShandon, Pittsburgh, Pennsylvania, USA), Turbitec® (Labonord, Templemars, France) ir *CytoSCREEN*® (Seroa, Monaco, Monaco). Mūsų pasirinkta sistema *Shandon PapSpin*™ yra paplitusi Vakarų Europoje. Pasaulinėje literatūroje publikuota keletas tyrimų, kuriuose analizuojamas *Shandon PapSpin*™ sistemos efektyvumas. Khalbuss ir kt., Weynand ir kt., Garbar ir kt., Rosenthal ir kt. analizavo *Shandon PapSpin*™ sistemos efektyvumą esant nustatytiems citologiniams gimdos kaklelio pakitimams. Jų darbuose *Shandon PapSpin*™ sistemos efektyvumas nebuvo įvertintas šiais atžvilgiais, t.y. jautrumo ir specifiškumo, vykdant atrankinės patikros programą, sistemos efektyvumo tiriant visą gimdos kaklelio medžiagą (pabrėžtina, kad jie tyrė dalytą gimdos kaklelio citologinę medžiagą), taip pat neapibrėžtų ir netinkamų tyrimų procento kitimo atžvilgiu.

Problemiška tai, kad *Shandon PapSpin* efektyvumo vertinimą riboja JAV vaistų ir maisto kontrolės patvirtinti reikalavimai technologijoms, taikomoms gimdos kaklelio citologinių tyrimų srityje, dėl ko neįmanomi tiesioginiai citocentrifuginių citologinių sistemų tyrimai.

Kadangi iš paskelbtų mokslinių publikacijų nėra aišku, koks yra *Shandon PapSpin*™ sistemos efektyvumas, kaip sumažėja netinkamų ir neapibrėžtų diagnozių procentas diagnozuojant ikivėžinę gimdos kaklelio patologiją, taip pat likusios po citologinio tyrimo

skystosios terpės tinkamumas ŽPV nustatyti PCR metodu, kai tiriama nedalyta medžiaga, tai mūsų atliktas darbas suteiks daugiau informacijos apie šią sistemą, jos taikymo galimybes vykdant gimdos kaklelio atrankinę patikrą, o žinios apie PGR reakcijos atlikimo galimybes leis spręsti apie šių ląsteles fiksuojančių terpių pritaikymą moksliniuose tyrimuose.

Kadangi ŽPV tipai, priklausantys didelės vėžio rizikos grupėms, yra susiję su gimdos kaklelio vėžio išsivystymu, todėl ankstyva lytinių takų infekcijos ir ikivėžinių pažeidimų diagnostika tapo aktuali ir reikšminga. Galimybė nustatyti žmogaus papilomos virusą iš likusios po citologinio tyrimo skystosios terpės yra papildomas citologinio tyrimo sistemų, kuriose naudojamos skystosiose terpėse fiksuotos gimdos kaklelio ląstelės, privalumas. Vieni jautriausių yra molekuliniai ŽPV genominės DNR identifikavimo metodai, iš kurių populiariausi ir plačiausiai naudojami yra polimerazės grandininė reakcija (PGR) bei įvairūs ŽPV DNR hibridizacijos metodai. Polimerazės grandininė reakcija (PGR) naudojama vykdant mokslinius tyrimus . 2005 metais Garbar ir kt. atliktame tyrime įvertintas *PapSpin*™ ir *Turbitec*® sistemų tinkamumas nustatyti ŽPV DNR hibridizacijos metodu *Hybride Capture* II. Tačiau lieka neaiškus šios sistemos tinkamumas PGR tyrimams, kurių nustatymas remiasi nedegeneruotos ŽPV DNR identifikavimu .

Tiriamieji ir tyrimo metodai

Rezultatai

Iš viso tyrimo laikotarpiu buvo atlikta 4945 citologiniai gimdos kaklelio tyrimai. 189 (6.4 %) moterims citologinis tyrimas leido nustatyti patologijos atvejus. Nedalytos medžiagos tyrimo fazėje 1500 moterims buvo atliktas dvigubas citologinis ištyrimas: 1500 gimdos kaklelio citologinių tepinėlių ir 1500 *PapSpin* preparatų. Dvigubo citologinio tyrimo rezultatai 104 (6,8%) moterims leido aptikti gimdos kaklelio patologiją. Daugiausia ikivėžinių CIN2/CIN3/CIS gimdos kaklelio pakitimų tyrimo laikotarpiu buvo nustatyta ir gydyta 30-40 metų amžiaus moterų grupėje, CIN1 patologija dažniau buvo diagnozuojama 30-34 metų amžiaus moterims.

Skirtingas patologijos atvejų procentas buvo gautas tiriant gimdos kaklelio ląsteles įprastinio tepinėlio ir *PapSpin* metodu. Įprastinio tepinėlio metodas leido aptikti 6% patologijos atvejų, *PapSpin* -5,3%. Dalytos medžiagos tyrimo fazės įprastinio tepinėlio ir *PapSpin* preparato tyrimų rezultatai pateikti 1 lentelėje.

1 lentelė. Dalytos medžiagos tyrimo fazės įprastinio tepinėlio ir *PapSpin* preparato tyrimų rezultatai

	I tyrimo fazė (n=1500)	
	Tepinėlis	*PapSpin*
Bethesda kategorija	(%)	(%)
Nėra pakitimų	1387 (92, 5)	1416 (94,5)
Tepinėlis netinkamas tirti	25 (1,7)	6 (0,4)
ASC-US	31 (2,1)	10 (0,7)
ASC-H	4 (0,3)	3 (0,2)
LSIL	20 (1,3)	29 (1,9)
HSIL	28 (1,9)	33 (2,2)
AGC	3 (0,2)	1 (0,1)
AIS	1 (0,1)	-

Plokščialąstelinė karcinoma	1 (0,1)	1 (0,1)
Skrandžio karcinomos metastazė	-	1 (0,1)
Iš viso	1500	1500

Dalytos medžiagos tyrimo fazėje didesnis ASC–US diagnozių skaičius buvo nustatytas įprasto tepinėlio metodu – 31 atv. (2.1 %) nei *PapSpin* metodu – 10 atv. (0.7 %) (p=0.001). Rezultatas statistiškai reikšmingas**.** Didesnio ASC-US diagnozės skaičiaus priežastys buvo mechaniškai suardytos ląstelės ruošiant įprastinį tepinėlį, džiūvimo ore artefaktai laiku nefiksavus tepinėlio, mažas atipinių ląstelių kiekis.

Didesnis LSIL atvejų skaičius buvo aptiktas tiriant gimdos kaklelio ląsteles *PapSpin* metodu – 29 atv. (1,9%), palyginti su 20 atv. (1,3%), nustatytų įprasto tepinėlio metodu, tačiau statistiškai patikimo skirtumo diagnozuojant šią patologiją nebuvo (p=0.12) Analizuojant įprasto tepinėlio ir *PapSpin* metodu mikroskopiniam tyrimui paruoštas ląsteles, paaiškėjo, kad geresnę LSIL atvejų diagnostiką *PapSpin* preparatuose sąlygojo optimali ląstelių fiksacija, leidžianti matyti pašviesėjimą apie ląstelių branduolį ir branduolio atipiją, kurią sąlygoja ŽPV infekcijos sukeltas ląstelių pažeidimas – koilocitai. 33 pav. pateiktos LSIL ląstelės, matomos tiriant įprastą tepinėlį, 34 pav. – LSIL ląstelės, matomos *PapSpin* preparate.

Dalytos medžiagos tyrimo fazėje įprastinio tepinėlio būdu nustatytas mažesnis HSIL ir piktybinių navikų atvejų skaičius – 28 (1.9%) nei *PapSpin* metodu –33 (2.2%), tačiau šis skirtumas nėra statistiškai reikšmingas (p=0,33). 35 ir 36 pav. matomi m Gimdos kaklelio citologinių tepinėlių ir *PapSpin* preparatų tyrimų rezultatų sutapimas matomas 8 lentelėje. 95% tyrimų rezultatų sutapo.

2 lentelė. Įprastinio citologinio tepinėlio ir PapSpin preparatų tyrimo rezultatų palyginimas

	Tepinėlis									
PapSpin	Tyrimui netinka	Nėra pakitimų	ASC-US	ASC-H	LSIL	HSIL	AGC	AIS	SCC	Iš viso *PapSpin preparatų*
Netinka tyrimui	6	-	-	-	-	-	-	-	-	6
Nėra pakitimų	18	1371	20	1	1	2	2	1	-	1416
ASC-US	1	7	2	-	-	-	-	-	-	10
ASC-H	-	1	1	1	-	-	-	-	-	3
LSIL	-	4	7	-	18	-	-	-	-	29
HSIL		2	1	2	1	26	1	-	-	33
AGC	-	1	-	-	-	-	-	-	-	1
Metastatinė skrandžio karcinoma	-	1	-	-	-	-	-	-	-	1
Plokščialąstelinė karcinoma	-	-	-	-	-	-	-	-	1	1
Iš viso tepinėlių	25	1387	31	4	20	28	3	1	1	1500

Apskaičiavus Kappa koreliacijos koeficientą patvirtintas 0,87 (95% PI:0.81-0.92) puikus abiejų metodų rezultatų sutapimas. Dalytos citologinės gimdos kaklelio medžiagos tyrimo fazėje 81 moteriai, dalyvavusiai mūsų tyrime, buvo atliktas gimdos kaklelio dalies chirurginis pašalinimas. Tiriamųjų moterų gimdos kaklelio citologinių ir histologinių tyrimų

palyginimas pateiktas 9 lentelėje. Dalytos medžiagos tyrimo fazėje įprasto tepinėlio ir *PapSpin* tyrimų jautrumas buvo 68.7% *vs* 78.1%, specifiškumas – 93.8% *vs* 91.8% atitinkamai. Teigiama tepinėlio ir *PapSpin* tyrimų prognozinė vertė buvo statistiškai reikšmingai neskirtinga – 88,0% ir 86,2%. Taip pat nenustatytas neigiamos tyrimo prognozinės vertės statistiškai reikšmingas skirtumas – 82,1 *vs* 86,8 %.

Atlikti 1450 *PapSpin* preparatų tyrimai. Visa medžiaga buvo tiriama PapSpin metodu. Citologinis *PapSpin* metodu gautų preparatų tyrimas leido aptikti 85 (5,9 %) patologijos atvejus. Citologinio tyrimo rezultatai, gauti tyrimo metu, pateikti 3 lentelėje.

3 lentelė. Citologinio tyrimo rezultatai, gauti nedalytos medžiagos tyrimo metu

	II tyrimo fazė (n=1445)
Bethesda kategorija	*PapSpin,* %
Nėra pakitimų	1352 (94,5)
Preparatas netinkamas tirti	8 (0,6)
ASC-US	20 (1,4)
ASC-H	10 (0,7)
LSIL	22 (1,5)
HSIL	31 (2,1)
AGC	2 (0,1)
AIS	-
Plokščialąstelinė karcinoma	-
Iš viso	1445

Nedalytos medžiagos tyrimo fazėje buvo aptikta 31 (2.1%) HSIL+ ir 22 (1.5%) LSIL atvejų skaičius, palyginti su dalytos medžiagos tyrimo fazės 28 (1,9) *vs* 20 (1,3). Mažas ASC-US ir ASC-H atvejų procentas – 1,4% ir 0,7% – iš esmės panašus kaip ir tiriant citologinę medžiagą dalytos medžiagos tyrimo fazėje *PapSpin* metodu – 0,7% ir 0,2% atitinkamai. Netinkamų preparatų procentas panašus kaip ir nedalytos medžiagos tyrimo fazėje – 0,6% ir 0,4% atitinkamai. Šis procentas tris kartus mažesnis nei netinkamų įprastinių tepinėlių dalytos medžiagos tyrimo fazėje.

Nedalytos medžiagos tyrimo fazės laikotarpiu buvo atlikta 60 gimdos kaklelio operacijų. Tiriamųjų moterų gimdos kaklelio citologinių ir histologinių tyrimų palyginimas pateiktas 4 lentelėje.

4 lentelė. Tiriamųjų moterų gimdos kaklelio citologinių ir histologinių tyrimų palyginimas nedalytos medžiagos tyrimo fazėje

	PapSpin						
Histologinė diagnozė	Nėra intraepitelinių pakitimų	ASC-US	ASC-H	LSIL	HSIL	Karcinoma	Preparatas netinkamas tirti
Gerybiniai pakitimai	15	1	2	5	1		1
CIN1			1	4			
CIN2		1	1		5		
CIN3/CIS		1	3	1	16		
Karcinoma					1		1

PapSpin tyrimo jautrumas – 75.9%, specifiškumas –96.5% Teigiama tyrimo prognozinė vertė – 95,7% , neigiama tyrimo prognozinė vertė 80,0%. Statistiškai reikšmingų skirtumų tarp pirmos tyrimo fazės įprastinio tepinėlio ir *PapSpin* preparato tyrimo jautrumo, specifiškumo, teigiamos ir neigiamos tyrimo prognozinės vertės nenustatyta ($p<0,05$).

Tolesniame tyrime naudojomės iš Valstybinio patologijos centro informacinės sistemos gauta informacija apie 48 (84%) moterų iš 58 operuotų stebėjimą po operacijos, susidedantį iš citologinių tepinėlių tyrimų ir pakartotinių konizacijų. Iš 48 stebimų moterų 11 (44 %) moterų, kurių operacinio pjūvio kraštuose buvo matoma CIN3/CIS (45,46 pav.), buvo atliktas pooperacinis citologinis tyrimas, kurio metu nustatytas HSIL, todėl jos pakartotinai konizuotos. 14 (56%) moterų histologinio tyrimo išvadose konstatuotas CIN3/CIS plitimas operacinio pjūvio kraštuose nepasitvirtino atliekant vėlesnius citologinius tyrimus (10 atvejų) ar pakartotinai konizavus (4 atvejai). Didesnė HSIL rizika pagal citologinio tyrimo rezultatus po konizacijos buvo susijusi su operacinio pjūvio kraštų būkle (12 lentelė). Ligos plitimo stebėjimo metu rizika buvo 20 kartų didesnė esant nustatytai displazijai operacinio pjūvio kraštuose (TS=20,0)

25 moterų operacinio pjūvio kraštai buvo pažeisti CIN3/CIS. 7 moterų buvo pažeisti endocervikinio ir giliojo operacinio pjūvio kraštai, 18 - endocervikinio operacinio pjūvio kraštas. 5 (34%) moterims, kurių buvo pažeisti endocervikinio pjūvio kraštai, pooperaciniu citologiniu tyrimu buvo nustatytas HSIL. 7 (85%) moterims, kurioms buvo pažeisti endocervikinis ir gilusis operacinio pjūvio kraštai, praėjus 4 mėn. po operacijos citologiniu tyrimu buvo nustatytas HSIL. Nustatyta ženkliai didesnė recidyvo rizika esant CIN3/CIS pažeidimui dviejuose operacinio pjūvio kraštuose (TS=161,0) nei viename (TS=10,5).

Tiriamųjų moterų *PapSpin* skystosios terpės su gimdos kaklelio medžiaga, likusia po citologinio tyrimo, buvo siunčiamos ŽPV nustatyti. Kaip *PapSpin* terpės kontrolė virusologiniam tyrimui, buvo siunčiamas ir naujos, nefiksuotos medžiagos mėginys PBS terpėje, atitinkantis *PapSpin* terpės numerį. Iš viso dėl žmogaus papilomos viruso buvo ištirti 176 mėginiai: 160 *PapSpin* terpės ir 16 naujai paimtos, nefiksuotos medžiagos mėginiai.

Tiriamųjų grupių DNR koncentracijų palyginimas. DNR buvo išskirta naudojant genominės DNR išskyrimo rinkinį (*Genomic DNA Purification Kit,* AB *Fermentas*) pagal gamintojo rekomendacijas. DNR išskirta kolonėlių metodu, tam naudojant genominės DNR išskyrimo rinkinį (*SorpoClean*^{TM} *Genomic DNA Extracion Module,* SORPO Diagnostics) pagal gamintojo rekomendacijas. Išskirtos DNR koncentracijos matuotos biofotometru (VU Gamtos mokslų fakulteto Genetikos katedroje) (*Ependorff*, Vokietija).Koncentracijų vidurkis pateikiamas 5 lentelėje

14 lentelė. DNR koncentracijų palyginimas tiriant *PapSpin* skystąją terpę ir nefiksuotą medžiagą

	DNR koncentracija, vidurkis((µg/ml)	DNR koncentracija, min (µg/ml)	DNR koncentracija, max (µg/ml)	±SD
PapSpin terpė (n =160)	**11,1**	**1,0**	**58,0**	**20,1**
Kontrolinis mėginys nefiksuota medžiaga (n=16)	**67,5**	**2,0**	**201,0**	**45,5**

Kaip matyti iš 14 lentelėje pateiktų duomenų matyti statistiškai reikšmingas skirtumas tiriant *PapSpin* skystąją terpę su citologine medžiaga ir nefiksuotos medžiagos mėginius. Koncentracija svyruoja nuo 11,1 µg/ml ir 67,5 µg/ml ($p<0,0001$). Vidurkis išskirtos DNR buvo taip pat didesnis nefiksuotoje medžiagoje nei *PapSpin* terpėje. Vadovaujantis gamintojo rekomendacijomis darome prielaidą, kad *PapSpin* skystojoje terpėje ląstelės buvo lizuotos ir DNR koncentracija nebuvo išskirta, skirtingai nei nefiksuotoje medžiagoje, kurioje fiksuojančių medžiagų nėra.

ŽPV DNR nustatymas tiriamosiose grupėse ŽPV DNR nustatytas tiriant išskirtą DNR. PGR metodu buvo pagausinta DNR, o pagausinti produktai, atlikus elektroforezę etidžio bromidu dažytame 2% agarozės gelyje, buvo analizuoti transiliuminatoriuje naudojant UV šviesos šaltinį (36 pav.). Žmogaus papilomos viruso DNR produktas yra 450 bazių porų ilgio. Atlikus ŽPV DNR nustatymą PGR metodu stebimas ryškūs nustatymo skirtumai *PapSpin* fiksuojančioje terpėje ir nefiksuotos medžiagos mėginyje. Gauti šie rezultatai: 5,6%(9/160) PapSpin skystosios terpės mėginių buvo nustatytas ŽPV, tuo tarpu kontrolinės grupės mėginiuose ŽPV buvo nustatyta 50%(8/16) ($p<0,000001$). ŽPV nustatymo rezultatai pateikti 6 lentelėje.

6 lentelė ŽPV nustatymas *PapSpin* skystojoje terpėje ir nefiksuotos medžiagos mėginiuose

	PapSpin terpė (n=160)		Nefiksuotos medžiagos mėginiai (n=16)		
	n	%	n	%	p
ŽPV teigiami	9	5,6	8	50,0	p<0,000001
ŽPV neigiami	151	94,4	8	50.0	

Pagal citologinio tyrimo rezultatus, kontrolinės nefiksuotos medžiagos grupės tyrimai, pasiskirstė sekančiai: 3atv. LSIL atvejai, 3 atv. HSIL atvejai ir 2 atv. ASC-H ir 8 nėra intraepitelinių pakitimų. 8 atv. (3LSIL,3HSIL,2ASC-H) buvo nustatytas ŽPV DNR.

IŠVADOS

1. Palyginus *Shandon PapSpin* sistema paruoštų preparatų ir įprastinio tepinėlio citologinių tyrimų diagnostinę vertę dalytos gimdos kaklelio medžiagos tyrimo fazėje paaiškėjo, kad *PapSpin* preparatų citologiniai tyrimai yra efektyvūs ir ne mažiau tikslūs nei įprastinių tepinėlių tyrimai, nors šioje tyrimo fazėje skystųjų terpių sistemai turėjo įtakos medžiagos dalijimas. *Shandon PapSpin* preparatų tyrimo jautrumas - 78,1%, įprastinio tepinėlio–68,7%, specifiškumas – 91,8% ir 93,8 % atitinkamai. Nors nustatyti skirtumai buvo statistiškai nereikšmingi ($p< 0,05$), geresnės efektyvumo tendencijos buvo nustatytos tiriant *Shandon PapSpin* preparatus. Nustatytas statistiškai reikšmingai mažesnis *PapSpin* sistema paruoštų netinkamų preparatų ir ASCUS išvadų kiekis nei įprastinio tepinėlio metodu: netinkamų tyrimų 6(0,4)% ir 25(1,7)% ($p=0,001$), ASCUS 10(0,7%) ir 31(2,1%)($p=0,001$).

2. *Shandon PapSpin* sistema iš skystosios terpės fiksuotos gimdos kaklelio medžiagos paruoštų preparatų citologiniu tyrimu galima tiksliai nustatyti ikivėžinius gimdos kaklelio ląstelių pakitimus. Tiriant nedalytą gimdos kaklelio citologinę medžiagą citologinio tyrimo jautrumas-75,9%, specifiškumas-96,5%, teigiama tyrimo vertė-95,7%, neigiama tyrimo prognozinė vertė-80,0%.

3. Nedalytos medžiagos *Shandon PapSpin* sistema paruošti preparatų tyrimai yra efektyvesni nei įprastinis tepinėlis. Tiriant *Shandon PapSpin* paruoštus preparatus gautas mažesnis ASCUS išvadų kiekis nei įprastinio tepinėlio metodu 20 atv.(1,4%) ir 31 atv.(2,1%) ir mažas netinkamų preparatų kiekis lyginant su įprastiniu tepinėliu 25 (1,7%) ir 8 (0,6%) atitinkamai.

4. Skystoji *Shandon PapSpin* terpė likusi po citologinio gimdos kaklelio tyrimo yra mažiau tinkama tirti ŽPV DNR PGR metodu MYO9/MY11 pradmenimis nei nefiksuota gimdos kaklelio medžiaga.

Trumpa informacija apie disertantę

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