

## THE LIMITS OF PAP SMEAR IN DIAGNOSING PREINVASIVE CERVICAL NEOPLASIAS

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**Keywords:** Pap smear, cytological smear

**Abstract:** The cervical-vaginal cytology can be interpreted after many classifications, starting from Babeș-Papanicolaou and ending with the Bethesda system, but no matter what the interpretation manner is it is really important to have a good quality of the smears and an experienced cytologist. If we refer to the total number of cases from the lot presented - 16732 cases, the results are satisfactory; 83% smears having normal limits, 10.14% MCB, 2.71% L-SIL, 0.82% H-SIL, 2.77% ASCUS, 0.10% AGC. In Iași there are significantly more patients with benign cell changes and L-SIL, while in Buzău there are significantly more patients with a normal smear, but there is also a higher frequency of the atypical squamous cells with an undetermined significance. Classically specialists say that 50-75% of the false negative results are due to the errors made when harvesting the sample. In conclusion we estimate that considering the present conditions in our country where the lack of interest and financial possibilities do not allow an action of detecting the lesions of the cervix organized nationwide, we can still make a selection of the cases with a high risk by making a free Pap smear for all the patients that come to a specialized service, either state or private, provided they respect the protocol of harvesting the cervical secretions, of preparing and reading/interpreting the smears.

### INTRODUCTION

In 1951 Willis defines neoplasia as “an abnormal mass of tissue that grows in excess when compared to the normal, cannot be controlled, is inconsistent with the growth of the normal tissues and it keeps growing progressively after the stimulus that caused it stops”. Subsequent research brought new elements that prove the complexity of mechanisms governing cell proliferation and differentiation as a result of clonal expansion. This cellular anarchy progresses in time and it finally leads to malignant cellular anarchy.

In 1971 Stein defines the malignant process from the genetic point of view: “cancer is a disease of gene adjustment, assuming the complex mechanisms that determine the gene expression in a given cell are malfunctioning, at a certain moment in its life cycle. The disorder of these mechanisms could allow gene depression or repression in a way that is not met in the cells that are normally differentiated” (1,5,6).

Among the feminine genital cancers, the cervical one is the easiest to detect, with small costs, benefiting from efficient methods of early diagnosis: cytology, HPV testing, colposcopy, biopsy. The detection of pre-cancer lesions by screening causes an important decrease of cervical cancer, especially for the advanced forms. It is known that the natural history of cervical cancer shows that the evolution of a pre-invasive lesion can take over 10 years to turn into a malignant lesion. The detection of these lesions and the application of the right treatment lead to their healing in almost 100% of the cases. Thus we can say that presently cervical cancer can be cured provided it is detected early (7,8,9).

The term of non-invasive cervical pathology is pretentious and difficult to define. In time, cytologists proposed different terms for denominating the minimum, severe and atypical changes that take place in the cell economy (18,19,20,21).

In 1968 Richart suggests the term of CIN with three stages: CIN 1 (mild dysplasia), CIN 2 (average dysplasia), CIN 3 severe dysplasia and CIS. A very important stage was to identify HPV and koilocyte in pre-cancer cells and it became a histologic marker of HPV infection (6,12).

In 1988 the National Institute for Cancer in USA replaces the term neoplasia, as being inappropriate for minor lesions, with SIL (squamous intraepithelial lesion) which he sub-classifies in L-SIL (low degree SIL, where we find HPV infection and CIN, the flat condyloma) and H-SIL (high degree SIL that includes CIN 2 and CIN3 with or without koilocyte), ASCUS with two subcategories ASC-H (H-SIL suspected atypical squamous cells) and ASC-US (the rest of the smears that define the most important cellular changes, but less important than the reactive changes and insufficient for diagnosing an intraepithelial squamous lesion) and CIN 3. The classification that is consensually called Bethesda System is challenged by those who think that the term SIL is not appropriate for the lesions with an increased evolutionary potential (2,3,4).

Everybody agrees that the first step in diagnosing the pre-invasive pathology of the cervix is the cytological smear. Being a logic and easy to do task at first sight, the routine harvesting of the smear at every routine specialized check up would be a great accomplishment, with a real diagnostic and prognostic impact (10,11,13).

## PURPOSE OF STUDY

Because this conduct is not a routine for the gynaecologic consult unless we are talking about speciality competitions, we chose 16732 patients for this work, all of them coming for a specialized consult in 5 specialized medical units in Iași and Buzău. We harvested cell samples for the Pap smear. The data was statistically processed in order to draw conclusions about the incidence of benign pathology of the cervix and the usefulness of detecting it by performing this test.(14,15,16,17)

## MATERIAL AND METHODS

Here we present a study we made on a sample of 16732 cases with cervical pathology where we harvested and performed the Pap smear, which we divided into 5 lots:

Lot 1 – 277 patients with cervical pathology consulted at the Family planning office within Elena Doamna Clinical Hospital of Obstetrics and Gynaecology Iași for contraceptive advice, representing 30,7% of all the consults;

Lot 2 – 1199 patients with cervical pathology, who were consulted in the ambulatory of Elena Doamna Clinical Hospital of Obstetrics and Gynaecology Iași;

Lot 3 – 963 patients with cervical pathology consulted in a private gynaecological practice in Iași;

Lot 4 – 838 patients with cervical pathology – pregnant in the first term of pregnancy that came for having an abortion at a specialized unit in Buzău;

Lot 5 – 13455 patients in Buzău, who came for a specialized consult because of various symptoms or for a routine check up and had a Pap smear.

We must also say that there is an error rate because the harvestings were performed by different doctors, so we cannot vouch for the correctness of the harvest and the processing and interpretation of the results was made in different labs.

The data was expressed in a way that allows it to be framed in categories; it was centralized in EXCEL and SPSS data bases and after that processed with statistical functions that are compatible.

## RESULTS

The distribution according to age groups shows the following aspects:

-lot 1 (277 cases) – the cervical pathology appeared mainly in the case of patients aged between 20-30 (52.7%), followed by those between 30-40 (31.4%);

-lot 2 (1199 cases) – we noticed the most increased frequency of cervical pathology for the patients aged between 30-40 (30.5%). followed by those between 40-50 (24.3%) and a frequency of 5.1% for the women over 60;

-lot 3 (963 cases) – cervical pathology is predominant for the age group 20-30 (42.4%);

-lot 4 (838 cases) – the most increased frequency of the patients with cervical pathology was noticed for the age group 30-40 (43.4%). followed by 20-30 year-olds (40.7%);

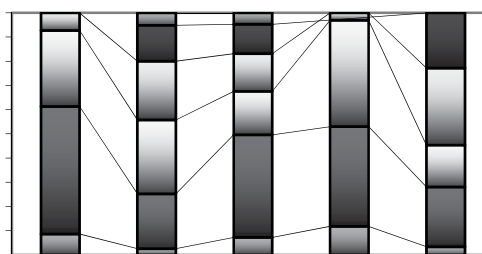
-lot 5 (13455 cases) – cervical pathology appeared mainly for the patients aged between 40-50 (31.8%).

**Table I. The distribution of the patients with cervical pathology on age group and lot of study**

Age group	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5	
	n	%	n	%	n	%	n	%	n	%
≤ 20	24	8.7	31	2.6	70	7.3	98	11.7	455	3.4
21-30	146	52.7	272	22.7	408	42.4	341	40.7	3324	24.7
31-40	87	31.4	366	30.5	173	18.0	364	43.4	2329	17.3
41-50	20	7.2	291	24.3	150	15.6	25	3.0	4284	31.8

51-60	-	-	178	14.8	117	12.1	-	-	3063	22.8
> 60	-	-	61	5.1	45	4.7	-	-	-	-
Total	277		1199		963		838		13455	

The lots were compared from the statistical point of view and we found out the following differences: lot 1 is characterized by a significantly higher frequency of cases with cervical pathology when compared with the other study lots; lot 2: the distribution of the patients with cervical pathology for the age groups 21-30 and 31-40 is significantly more reduced when compared with lot 3; lot 3 is characterized by almost similar shares of cervical pathology for the age groups 31-40, 41-50 and 51-60; for lot 4 we notice that cervical pathology is predominant for the patients in the age group 31-40; lot 5 is characterized by significantly more increased cases of cervical pathology in the patients over 50.



■ < 20 ■ 21-30 ■ 31-40 ■ 41-50 ■ 51-60 ■ > 60 ani

Fig. 1. The structure of the lots according to the patients' age groups

**Table II. The matrix of correlation of the cases with cervical pathology on age groups**

Correlations	Lot 1	Lot 2	Lot 3	Lot 4
Lot 2	$\chi^2=180.25$ $p<0.001$	-		
Lot 3	$\chi^2=81.80$ $p<0.001$	$\chi^2=147.47$ $p<0.001$	-	
Lot 4	$\chi^2=25.15$ $p<0.001$	$\chi^2=452.75$ $p<0.001$	$\chi^2=321.53$ $p<0.001$	-
Lot 5	$\chi^2=252.15$ $p<0.001$	$\chi^2=841.71$ $p<0.001$	$\chi^2=923.63$ $p<0.001$	$\chi^2=897.78$ $p<0.001$

The average age for the study lots was significantly more increased for lots 2 and 5 when compared to the other lots of study:

**Table III. The matrix of correlation of cervical pathology on age groups**

Correlations	Lot 1	Lot 2	Lot 3	Lot 4
Lot 2	$t=10.58$ $p<0.001$	-		
Lot 3	$t=0.04$	$t=16.20$	-	

Lot 4	p>0.05 t=0.90	p<0.001 t=14.33	t=1.48	-
Lot 5	p>0.05 t=12.71 p<0.001	p<0.001 t=1.0 p>0.05	t=21.83 p<0.001	t=25.46 p<0.001

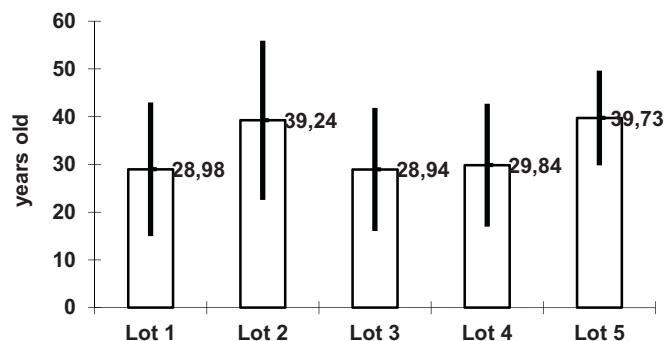


Fig. 2. The average age of the patients with cervical pathology on lots of study

When we compare these two geographic areas we notice that there are significantly more patients with cervical pathology in Iasi for the age groups 21-30 and 31-40, and for Buzău there is an increased frequency for the age groups 41-50 and 51-60 ( $\chi^2=934.95$ ;  $p<0,001$ ). The average age registered in the two areas was significantly higher in Buzău ( $t=7.22$ ;  $p<0,001$ ).

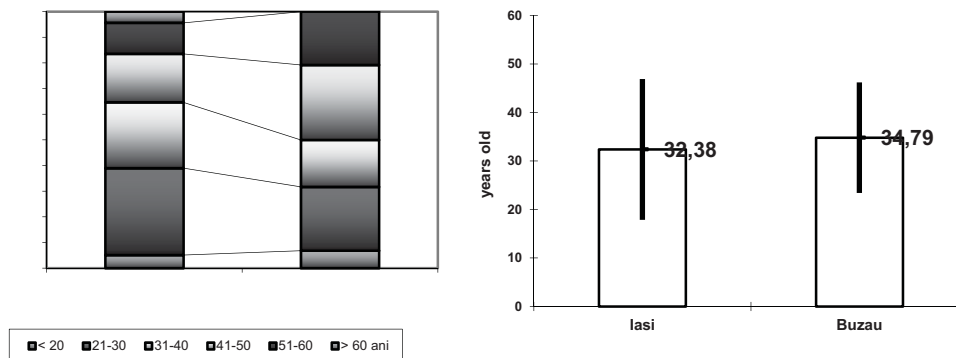


Fig. 3. The distribution on age groups compared for the two geographic areas

**Distribution on the area the patients come from**

The patients with cervical pathology came mainly from the urban area for all the lots, except those in lot 5, where we observed more patients coming from the rural area.

Table IV. The distribution of the patients with cervical pathology according to the area they come from on lots of study

Area	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5	
	n	%	n	%	n	%	n	%	n	%

Urban	193	69.7	873	72.8	836	89.6	554	66.1	5436	40.4
Rural	84	30.3	326	27.2	127	10.4	284	33.9	8019	59.6
Total	277		1199		963		838		13455	

When we compared the two geographic areas we discovered that in Iași the patients came mainly from the urban area, while in Buzău they came from the rural area.

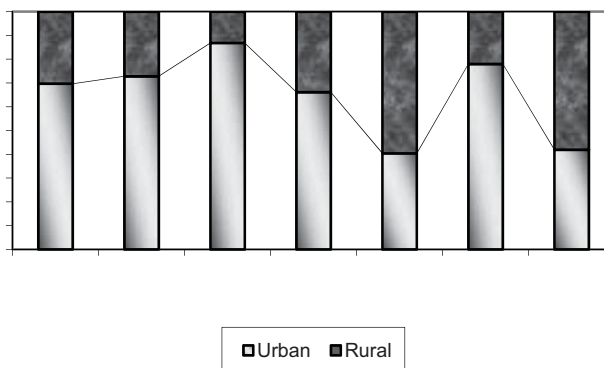


Fig. 4. Structure of the lots depending on the area they come from

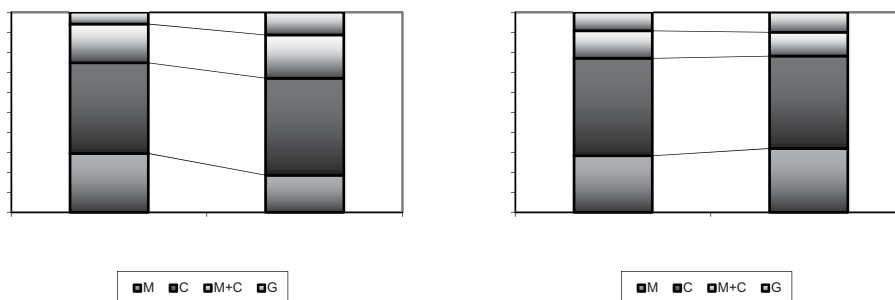
Table V. Matrix of correlation of cases with cervical pathology depending on the areas they come from and geographic areas

Correlations	Lot 1	Lot 2	Lot 3	Lot 4	Buzău
Lot 2	$\chi^2=0,90$ $p>0,05$	-			
Lot 3	$\chi^2=43,53$ $p<0,001$	$\chi^2=62,37$ $p<0,001$	-		
Lot 4	$\chi^2=1,04$ $p>0,05$	$\chi^2=10,24$ $p<0,001$	$\chi^2=107,86$ $p<0,001$	-	
Lot 5	$\chi^2=94,95$ $p<0,001$	$\chi^2=470,32$ $p<0,001$	$\chi^2=2132,11$ $p<0,001$	$\chi^2=785,70$ $p<0,001$	
Iași					$\chi^2=1086$ $p<0,001$

#### Distribution according to the cytobacteriological smear

Only lots 1 and 2 in Iași had an interpretation of the cytobacteriological smears (FCB).

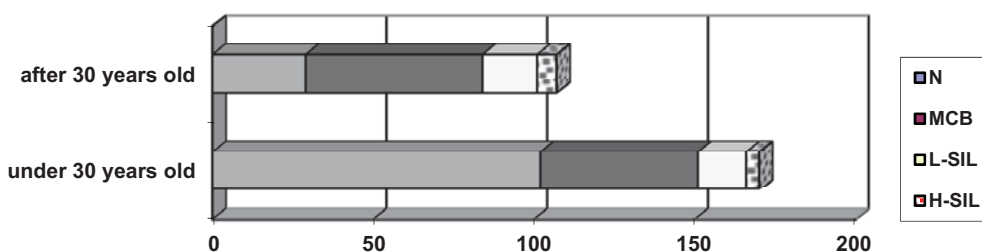
We noticed a greater presence of the coccobacillary flora for both lots of study, without recording other significant differences depending on the patient's age.



**Fig. 5. Distribution of the patients according to the cytobacteriological smear for the lots in Iași**

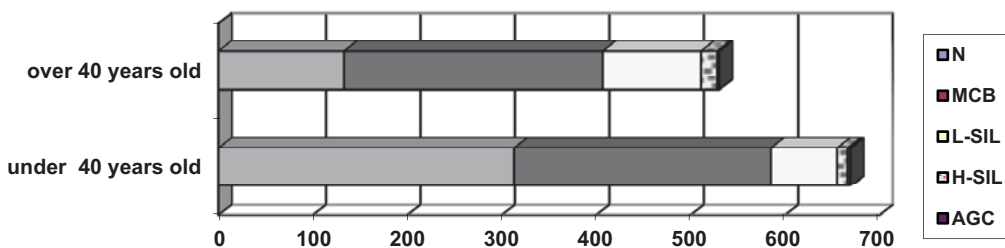
**Distribution depending on cytodiagnosis**

For lot 1.60% of the patients under 30 years old had a normal smear, while for the patients after 30 years old the benign cell changes are predominant (51,4%) ( $\chi^2=28.71$ ;  $p<0,001$ ).



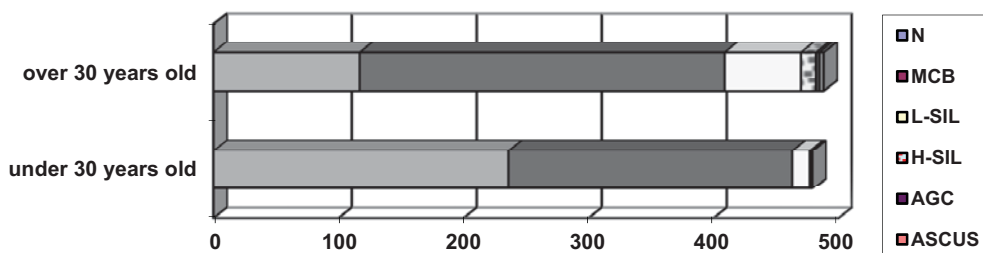
**Fig. 6. Distribution of the patients in lot 1 depending on cytodiagnosis**

For lot 2.47% of the patients under 30 years old had a normal smear, while for the patients after 30 years old the benign cell changes are predominant (51.7%) ( $\chi^2=65.55$ ;  $p<0.001$ ).



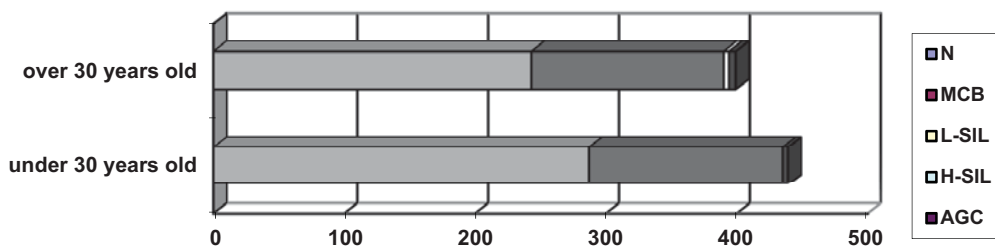
**Fig. 7. Distribution of the patients in lot 2 depending on cytodiagnosis**

For lot 3, 4.9% of the patients under 30 years old had a normal smear and 47% with benign cellular changes, while for the patients after 30 years old the benign cell changes are predominant (at 59.8%) and 12.5% of the patients with L-SIL ( $\chi^2=90.66$ ;  $p<0.001$ ).



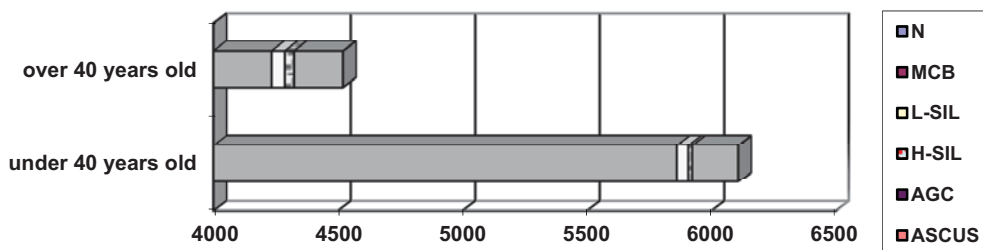
**Fig. 8. Distribution of the patients in lot 3 depending on cytodiagnosis**

For lot 4, both age groups had mainly normal smears (over 60%), and there were benign cellular changes in 34% of the patients under 30 years old and in 37% of the patients over 30 years old, without recording significant changes depending on the age group ( $\chi^2=5.87$ ;  $p=0.118$ ).



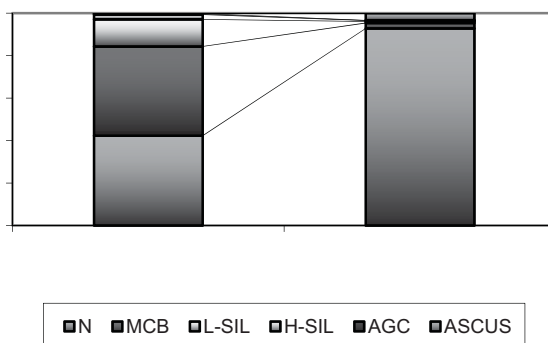
**Fig. 9. Distribution of the patients in lot 4 depending on cytodiagnosis**

For lot 5, 5.96% of the patients under 40 years old and 94% of the patients over 40 years old had a normal smear, and L-SIL and H-SIL lesions had higher frequencies for the patients over 30 years old ( $\chi^2=35.20$ ;  $p<0.001$ ).



**Fig. 10. Distribution of the patients in lot 5 depending on cytodiagnosis**

When we compared the geographic regions, we noticed that in Iași we registered significantly more patients with benign cellular changes and L-SIL, while in Buzău we registered significantly more patients with normal smears, but we also notice the increased frequency of atypical squamous cells with undetermined significance ( $\chi^2=4934.17$ ;  $p<0.001$ ).



**Fig. 11. Distribution depending on cytodiagnosis made comparatively on geographic areas**

The profile of the patient with cervical pathology on lots of study

Parameter	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5
Age	30 years old	40 years old	30 years old	30 years old	40 years old
Area of origin	urban	urban	urban	urban	rural
Job status	employed	-	-	-	-
Cytobacteriological smear	coccobacillus	coccobacillus	-	-	-
Cytodiagnosis	MCB	MCB	MCB	Normal	ASCUS
Diagnosis	cervicitis	cervicitis	cervicitis	-	-

The profile of the patient with cervical pathology on residential areas

Parameter	Iași	Buzău
Age	32 years old	35 years old
Area of origin	urban	rural
Cytobacteriological smear	coccobacillus	-
Cytodiagnosis	MCB	ASCUS
Diagnosis	cervicitis	-

## DISCUSSIONS

The cervical-vaginal cytology can be interpreted after many classifications, starting from Babeș-Papanicolaou and ending with Bethesda system, but no matter what the interpretation manner might be it is very important to consider the quality of the smears and the cytologist's experience. The cytologist will have to be up to date, will need competence and also moral and professional



qualities that can guarantee the quality of the work done. We appreciate that 50-75% of the false negative results are due to the errors appeared when harvesting the smear.

We remind you that the harvesting devices recommended are the Ayre wooden or plastic spatula (preferably for the exo-cervix and the posterior vaginal cul de sac) and the Cervex-Brush for the endo and exocervix. You must also respect all the harvesting conditions: the previous treatment of the inflammatory or atrophic local processes, no sexual intercourse, vaginal washing or vaginal digital examination 48 hours before harvesting. Harvesting must be performed after applying a speculum or two valves (they must necessarily be held by a help) and you must highlight the cervix without hurting it. You should perform the first harvest from the exo-cervix surface and from the posterior cul de sac, in order to avoid a possible contamination with blood (when you harvest the sample with the brush from the endo and exo-cervix). The harvested secretions are spread on separate slides for the endocervix, exocervix and the posterior cul de sac, then they are fixed by using immersion in 95% alcohol or a fixing spray with polyethylene - glycol (air drying, as it is habitual, causes a cell ratatination). The recommended coloration is Papanicolaou, but the routine one is Way-Grumwald Giemsa. (Mc.Googan). Of course, you must register and fill in a form that will be sent to the lab.

Presently the use of ThinPrep or Cyto-Rich technique of preparing the slides in liquid, which is also called the technique of “monolayer or thin layer display”, which increases the sensitivity and specificity of the Paptest (Badea), is not possible on a large scale, because of the costs and also of the lack of cyto-technicians and trained specialists. Moreover, authors like Schneider think that the future belongs to the automatic processing and interpretation of the smears.

The lot of study presented here causes a series of comments:

The sample of 1115 cases (lot 1 and 4) who came for a contraceptive or contragestive consult, within the context of family planning, were young patients, who did not have any relevant symptoms, but who were diagnosed during the consult with a cervical pathology that was unknown to them in 44,8% of the cases (contraception 52.8%, contragestion 36.8%);

The sample of 14554 cases (lot 2 and 5), who came in the ambulatory of some specialized units with different genital symptoms, for a consult, showed a percentage of 5% cellular anomalies in lot 5 compared to 62.9 % in lot 2. The difference is hard to explain. It could be caused by the deficiencies in harvesting the samples and preparing the slides; the sample of 963 cases (lot 3) came for a consult because of various genital and breast symptoms. During the first consultation they had cells harvested for the Pap smear or, depending on the case, this happened during the second consult if they needed a preparation of the vagina or they had to respect the conditions for harvesting, preparing and reading the smears and also the filling in of a questionnaire. We detected 63.4% smears with a changed cytology: MCB-53.8%, L-SIL-7.78%, H-SIL 1.24% and ASCUS-0.41%.

## CONCLUSIONS

If we report to the whole presented lot (16732 cases), the results are satisfactory; 83% smears being within normal limits, 10.14% MCB, 2.71% L-SIL, 0,82% H-SIL, 2.77% ASCUS, 0.10% AGC.

So we can afford saying that a significant number of smears that belong to the category “smear within normal limits” are false negative for various reasons and the results for lot 3 are the closest to reality, but, of course, the number of cases is not representative.

We appreciate that, considering the fact that the interest and financial possibilities in our country do not allow an action of detecting the cervical lesions that would be organized nationwide, we can make a selection of the cases of risk by performing a free Pap smear for all the patients addressing a specialized state or private clinic or office, provided they respect the protocol of harvesting the cervical secretions, of preparing and reading the slides

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