A FOLLOW-UP STUDY IN A GROUP OF WOMEN WITH ASCUS/ASC-H CYTOLOGY: PREVALENCE OF CARCINOGENIC HPV AND CLINICAL EVOLUTION

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Abstract: *Cervical cancer is a public health problem worldwide, in European Union and in Romania. This observational study aimed to investigate the evolution of clinical status and HPV infectivity in a cohort of women with ASCUS or ASC-H cytology, monitored in a clinic of gynaecology from Bucharest. We found more severity of colposcopic and histopathologic lesions in ASC-H women compared to ASCUS ones at enrolment, but a comparable clinical evolution after two years of follow-up. As regard of HPV infection, testing had limited availability. In cases of testing available, high prevalence of HPV 16, 51, 32, 52 and 18 in ASCUS group and 16, 31, 18 in ASC-H group was found.*

INTRODUCTION

Despite its large potential of prevention through primary and secondary public health interventions, cervix cancer still represents a major public health problem worldwide, in European Union and in Romania.(1) It affects annually more than half million women worldwide, most of them at active age (highest frequency between the ages of 35 and 50).(1,2) In European Union, EUCAN estimates for 2012 showed the occurrence of 33354 new cases (6.3% of the global cases), 12996 deaths (5% of the global deaths) and a five-year prevalence of 114136 cases.(3) Although the burden of cervix cancer is less detrimental in European Union compared to other regions, this disease remains a reason of major inequalities in health even in EU, with wide variations in incidence and mortality among the member states. In 2014, there was 7:1 ratio between highest and the lowest incidence of cervix cancer in EU member states (31.5 versus 4.2 new cases/100000 inhabitants in Romania and Malta respectively) and 10:1 ratio in standardised mortality rates (11.9 versus 1.3 deaths/100000 inhabitants in Romania and Finland respectively).(4) Average EU incidence and standardised mortality rates in same year reached to 11.7 new cases per 100000 inhabitants and 3.1 deaths per 100000 inhabitants.(4) Most detrimental situation is seen by far in Romania, with over 3,500 new cases and 2400 deaths per year.(4,5)

Main cause of cervix cancer is the persistent infection with some high-risk genotypes of Human Papillomavirus (HPV), most cited being 16/ 18/ 31/ 33/ 35/ 39/ 45/ 51/ 52/ 56/ 58/ 59/ 66/ 68 (6).(6-10) Genotypes 16 and 18 are most common and induces around 71% of all cervical cancers worldwide.(11) The HPV infections are most common viral infections of the reproductive tract and majority of them are asymptomatic and solve spontaneously in one - two years.(6) Only 5-10% of these infections are persistent, and among them, a small proportion may lead to pre-cancerous lesions and even fewer induce invasive carcinoma after a time lag of 20 years or longer.(6)

Vaccination against Human Papillomavirus (HPV) is the main primary prevention alternative, and three vaccines are available: the quadrivalent vaccine (6, 11, 16, 18) licensed in 2006, the bivalent vaccine (16, 18) licensed in 2007 and the nonavalent vaccine (6, 11, 16, 18, 31, 33, 45, 52 and 58) licensed in 2014.(6) Till March 2017 71 countries worldwide have introduced HPV vaccination for girls in their vaccination programs.(12)

Screening for cervix cancer or for HPV infections are most common alternatives for secondary prevention (early detection).(13) Populational screening for cervix cancer is recommended by World Health Organization for all women aged 30 or more, every 5 to 10 years if the screening test is negative and depending on the test used, but at least once during lifetime for all women aged 30 – 49.(13) In EU, indication for screening for cervix cancer is wider, using pap smear in women not before the age of 20, but no later than the age of 30.(2) This indication has been enforced in 2003 through a Recommendation of the Council of the European Union which urges the member states to implement systematically populational screening programs for cervix cancer (also for breast and colon cancer), by organizing a call/recall system, quality assurance mechanisms at all levels and effective and appropriate diagnosis, treatment and after-care service, upon evidence-based guidelines.(14) Despite this recommendation has been issued from more than one decade, last implementation report suggests that population-based screening programmes are implemented with certain heterogeneity in 22 member states nationally or regionally (Romania is mentioned as implementing population-based screening programme, still in process of rolling out).(15) The screening test is cytology, and the screening interval is 3 or 5 years.

Screening against HPV infection has been proven to provide a 60-70% higher protection against cervical invasive carcinoma compared to cytology (16). It was reported as being offered in seven countries (Denmark, Finland, Italy, Sweden, Malta, Portugal and Romania – in co-testing with cytology).(15)

Despite the encouraging data about the availability of screenings for cervix cancer and HPV in Romania, a coverage of 14.2% of the eligible population (all women 25 – 64 years) was

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reported for cytology during 2012-2015 and no data are available for HPV testing.(17) More than this, according to the legal framework of the screening programme, cytology is free of charge (if performed within the programme), but HPV testing is not covered by the programme, meaning that the cost of the HPV screening should be paid directly by the client, this limiting the access to the testing due to affordability reasons. All these results suggest in fact a limited access of the eligible population to cytology and HPV screening. In this context, the detection of the cervix cancers is mainly based on opportunist or passive approaches, which limits the outcome of the treatment and the progress in decreasing mortality. The therapeutic approach remains the only tool for obtaining the best possible outcome. In case of low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) cytology results, the most relevant clinical guidelines are unequivocal, but recommendations have slight heterogeneity when it comes to Atypical squamous epithelial cells of uncertain significance/Atypical Squamous Cells, Cannot Rule Out High-Grade Squamous Intra-epithelial Lesion (ASCUS/ASC-H) (most recommended options being follow-up with repeat cytological examination, human papillomavirus (HPV) DNA testing and immediate colposcopy.(2,19,20). The women with ASCUS and ASC-H cytology have low probability to develop invasive cervix cancer (0.1 to 0.2%), but 5 to 17% and 24% to 94% of cases with ASCUS and ASC-H respectively are expected to have high-grade precancerous lesions (cervical intraepithelial neoplasia (CIN) grades 2 and 3).(21) There is a consensus that HPV screening is the most effective strategy for the management of women with ASCUS /ASC-H cytology.(19)

PURPOSE

Our research is focused on the evolution of clinical status and HPV infectivity in a cohort of women with ASCUS or ASC-H cytology, monitored in a clinic of gynaecology from Bucharest.

MATERIALS AND METHODS

We performed a prospective study, consisting in two years follow-up of a cohort of women with cervical abnormalities, seeking specialty gynaecologic services in a clinic of gynaecology from Bucharest during a five years period (since 1st of January 2010 – 31st of December 2014). This paper is focused only on 345 women with ASCUS or ASC-H cytology at enrolment, but results at enrolment for overall cohort were published elsewhere.(22) This study is observational and it has been approved by the Ethical Committee of the Clinic.

All women were enrolled in the study, based on their informed consent. They were examined based on the following protocol:

a. At enrolment: cytology, colposcopy and recommendation for HPV testing (charged);
b. Follow up at every 6 months, consisting in cytology and colposcopy.
c. Treatment according to American Society for Colposcopy and Cervical Pathology (ASCCP).(19) In case of persisting abnormal cytology (persistent/evolutive lesions) or persisting/evolutive colposcopic lesions or persistent HPV infection, the treatment included excision of the cervical lesions and histopathological exam.
d. In women with HPV infection, repeated HPV testing annually. However, the HPV testing was not available free of charge (not included in the package of medical services), so patients should pay 100% of the tests cost. This issue limited the access to testing, due to affordability.

Collected data included personal characteristics, clinical status at enrolment (cytology, colposcopy, HPV testing) and histopathological examination.

Cytology results were interpreted following the Bethesda reporting system as: NILM (Negative for Intraepithelial Lesion or Malignancy), ASCUS (Atypical squamous epithelial cells of uncertain significance), ASC-H (Atypical Squamous Cells, Cannot Rule Out High-Grade Squamous Intra-epithelial Lesion), LSIL (Low-grade squamous intraepithelial lesion) and HSIL (high-grade squamous intraepithelial lesion).(23)

Colposcopy results were classified as: normal (without lesions), atypical transformation grade 1 (ATG1) and grade 2 (ATG2) areas.(24)

HPV testing was considered positive if any type of HPV was revealed. We followed in particular the presence of any of the fourteen known high risk genotypes 16/18/31/33/35/39/45/51/52/56/58/59/66/68, as single infection or coinfection.

The histopathologic examination was classified as: CIN1 (low grade lesion), CIN2, CIN3, CMI (micro invasive carcinoma) and CI (invasive carcinoma).(25)

Data analysis: The subgroups ASCUS and ASC-H were analysed as personal characteristics, type of colposcopy lesions, presence of HPV infection and type of high risk antigens and type of histopathologic diagnosis, if available. The analysis has been performed at two moments: T0 (at enrolment visit) and T1 (at the end of the two-year follow-up period). We planned the follow-up visits every six months, but the patients came irregularly. However, due to the observational characteristic of the study, we considered for analysis all the cases, even those with incomplete follow-up. In these cases, we considered the status at the last visit as final status at T1.

The scale variables were analysed as mead±SD or median and interquartile range (IQR). They were assessed for normality using the Kolmogorov-Smirnov test. A p-value <0.05 was considered for statistical significance (two tailed test). Categorical data were presented as proportions with one decimal. Comparisons between subgroups were performed using T student test or Mann Whitney U for scale variables and Chi square test or Fisher exact test for categorical variables ($p<0.05$). Statistical analyses were performed using SPSS 23.0.

RESULTS

We investigated 345 women with ASCUS or ASC-H cytology, which represented 47.2% from our initial cohort. ASCUS cases seemed to be far more frequent, with a 5:1 ratio compared to ASC-H ones, but no other significant differences in personal characteristics were found between the two groups (table no. 1).

However, both groups are different compared to general female population, having higher representation of urban environment and high education (55.1% and respectively 26.8% in general population).(26,27)

Table no. 1. Personal characteristics of ASCUS and ASC-H groups

Characteristic	ASCUS group	ASC-H group	p-value
No (%)	287 (83.2)	58 (16.9)	NA
Age (years)			
Mean±SD (min; max)	33.2±7.50 (19, 55)	35.7±8.23 (22, 62)	NA
Median	33	35	0.087*
IQR	11	11.3	NA
Height (cm)			
Mean±SD (min; max)	165.8±5.01 (151; 178)	159.4±13.69	NA

Median	165	165	0.260*
IQR	7	6.3	NA
Weight (kg)			
Mean±SD (min; max)	61.2±8.64 (45; 102)	63.6±16.80 (46; 159)	NA
Median	60	60	0.949*
IQR	12	12.3	NA
Education (no, %)			
Low	7 (2%)	1 (2%)	0.943**
Medium	72 (25%)	15 (26%)	
High	208 (72%)	42 (72%)	
Residence (no, %)			
Urban	260 (90.6%)	53 (91.4%)	0.542**

** Mann Whitney U test; **Chi square test*

Clinical status at enrolment is revealed in table no. 2.

Table no. 2. Clinical status at enrolment for ASCUS and ASC-H groups

Characteristic	ASCUS group	ASC-H group	p-value
Colposcopy results – no (%)			
Normal	35 (12.2%)	2 (3.4%)	<0.001**
ATG 1	218 (76.0%)	24 (41.4%)	
ATG 2	34 (11.8%)	32 (55.2%)	
HPV testing – no (%)			
Performed	217 (75.6%)	51 (87.9%)	0.040**
Pos. result*	189 (87.1%)	44 (86.3%)	0.87**
HR antigens-any*	170 (78.3%)	42 (82.4%)	0.13**
HR antigens – associations*	119 (54.8%)	11 (21.6%)	<0.001**
HR antigens-single*	51 (23.5%)	31 (60.8%)	
HPV 16	58 (26.7%)	19 (37.3%)	0.068**
HPV 18	20 (9.2%)	6 (11.8%)	0.29**
HPV 31	28 (12.9%)	6 (11.8%)	0.41**
HPV 33	10 (4.6%)	2 (3.9%)	0.99***
HPV 35	10 (4.6%)	2 (3.9%)	0.99***
HPV 39	8 (3.7%)	0 (0%)	NA
HPV 45	6 (2.8%)	0 (0%)	NA
HPV 51	30 (13.8%)	1 (2.0%)	0.008***
HPV 52	27 (12.4%)	1 (2.0%)	0.016***
HPV 56	8 (3.7%)	1 (2.0%)	0.46***
HPV 58	6 (2.8%)	3 (5.9%)	0.23***
HPV 59	9 (4.1%)	2 (3.9%)	0.65***
HPV 66	15 (6.9%)	0 (0%)	NA
HPV 68	6 (2.8%)	0 (0%)	NA
Histopathologic examination – no (%)			
Performed	77 (26.8%)	40 (69.0%)	<0.001**
CIN1	22 (28.6%)	2 (5.0%)	0.003**
CIN2	51 (66.2%)	26 (65%)	0.89**
CIN3	4 (5.2%)	11 (27.5%)	<0.001**
CMI	0	0	NA
CI	0	1 (2.5%)	NA

**proportion calculated from performed tests (n=217 and 51 for ASCUS and ASC-H respectively); **Chi square test; ***Fisher exact test*

We found a significant difference in severity of the colposcopic lesions between the two subgroups. Women with ASC-H cytology had ATG 2 in a much higher proportion (55.2 versus 11.8%).

The HPV testing was recommended to all women, but it was available in 77.7% of the cases. Proportion of women which performed the HPV testing was significantly higher in ASC-H group, probably due to a higher awareness and concern about the severity of their lesion. Among the tested women, we found similar proportion of infection (87.1 and 86.3% in ASCUS and ASC-H respectively). Also, most of the tested cases have at least one high risk antigen (78.3 and 82.4% in ASCUS and ASC-H respectively) and no statistical difference was found between groups. High risk HPV types seemed to be prevalent in associations for the ASCUS group, but in single infections in ASC-H.

HPV types 16, 51, 31, 52 and 18 occurred most frequently in ASCUS group, but in ASC-H group, types 16, 18 and 31 were found most often. Despite the fact that HPV 16 occurred with higher frequency in ASC-H group (37.3% versus 26.7%), this difference did not meet the statistical significance in our study. The only statistically significant difference was found for types 51 and 52, which occurred more frequently in the ASCUS group.

Histopathologic examination at enrolment has been performed in significantly higher proportion in the ASC-H group. Among the examined samples, CIN1 lesions occurred in significantly higher proportion in the ASCUS group, but CIN 3 occurrence was significantly higher in ASC-H group (table no. 2).

All the cases were planned to be followed for two years, but some of them missed some visits or came for follow-up irregularly. Details about the number of visits are shown in table 3, but no statistical difference was found between groups (p=0.50).

Table no. 3. Number of visits per group

No of visits	ASCUS		ASC-H		Total	
	no	%	no	%	no	%
1	21	7.3%	3	5.2%	24	7.0%
2	5	1.7%	2	3.4%	7	2.0%
3	15	5.2%	1	1.7%	16	4.6%
4	246	85.7%	52	89.7%	298	86.4%
Total	287	100.0%	58	100.0%	345	100.0%

24 women (7%) came only for the first visit and these cases were excluded from the final analysis. All the others were analyzed considering their last follow-up visit as final. Among them (n=321), 2.5% and 11.5% had a period of observation shorter than 12 and 18 months respectively. The median follow-up lasted 712 days, the shorter 220 days and the longer 979 days.

After two years of observation, 57.0% of the women had normal cytology, 28.3% and 3.7% had ASCUS and ASC-H respectively, but 9.3% and 1.6% had LSIL and HSIL respectively. The two groups had similar evolution (p=0.65, Fisher exact test, details in table no. 4).

Table no. 4. Final cytology in ASCUS and ASC-H groups

Cytology at T1	ASCUS		ASC-H	
	No	%	No	%
NILM	147	55.3%	36	65.5%
ASCUS	80	30.1%	11	20.0%
ASC-H	10	3.8%	2	3.6%
LSIL	25	9.4%	5	9.1%
HSIL	4	1.5%	1	1.8%
Total	266	100.0%	55	100.0%

The colposcopy showed normal results in 45% of cases and ATG1 and ATG2 in 49% and 6% of women, again, without significant difference among groups (p=0.19, Chi square

test, table no. 5.

Table no. 5. Final colposcopy in ASCUS and ASC-H groups

Colposcopy at T1	ASCUS		ASC-H	
	No	%	No	%
CN	116	43.6%	30	54.5%
ATG1	133	50.0%	24	43.6%
ATG2	17	6.4%	1	1.8%
Total	266	100.0%	55	100.0%

Actually, 109 and 19 women from ASCUS and ASC-H group respectively had a less severe lesion at colposcopy after two years and 25 and 1 respectively in ASCUS and ASC-H groups had a more severe lesion, the rest maintaining the status from the enrolment.

The situation was similar per groups (p=0.09, Chi square test, details in table no. 6).

Table no. 6. Colposcopy at T0 and T1 per group

ASCUS T0				
	CN	ATG1	ATG2	Total
CN	18	13	0	31
ATG1	82	109	12	203
ATG2	16	11	5	32
Total	116	133	17	266
ASC-H T0				
CN	1	0	1	2
ATG1	10	13	0	23
ATG2	19	0	11	30
Total	30	13	12	55
Legend:	*Improvment*	*Stationar*	*Progression*	

During the observation period, another 120 women have performed histopathologic exams (38.7% and 30.9% of ASCUS and ASC-H women, with no statistically significant difference between groups, p=0.28, Chi square test). Detailed results are shown in table no. 7.

Table no. 7. Final histopathology in ASCUS and ASC-H groups

Histopathology at T1	ASCUS		ASC-H	
	No	%	No	%
CIN1	13	12.62%	3	17.6%
CIN2	77	74.76%	11	64.7%
CIN3	11	10.68%	2	11.8%
CI	0	0.00%	1	5.9%
Other	2	1.94%	0	0.0%
Total	103	100.00%	17	100.0%

p=0.27, Fisher exact test

Overall, majority of the women (259 and 55 women from ASCUS and ASC-H) did perform at least one HPV testing, either at enrolment or during the observation period. 153 and 28 women from ASCUS and ASC-H groups were tested or re-tested during the follow-up visits. Most of them had one test, but in 34 cases two or three tests were performed.

Considering the last known testing for each of the cases that were followed (including for those tested only at enrolment), the epidemiologic situation of the women at the end of the study is presented in table no. 8.

Table no. 8. HPV status in ASCUS and ASC-H groups

HPV	ASCUS		ASC-H		
	No	%	No	%	p-value
Performed	196	73.7%	35	63.6%	0.13**
Positive*	176	89.8%	32	91.4%	0.99**
HR HPV*	155	79.1%	31	88.6%	0.19**
HR HPV-Association*	52	26.5%	9	25.7%	0.62**
HR HPV-single*	103	52.6%	22	62.9%	
HPV 16	51	26.0%	15	42.9%	0.04**
HPV 18	13	6.6%	2	5.7%	0.99***
HPV 31	22	11.2%	4	11.4%	0.93***
HPV 51	29	14.8%	1	2.9%	0.07***
HPV 52	23	11.7%	1	2.9%	0.18***

**proportion calculated from performed tests (n=196 and 35 for ASCUS and ASC-H respectively); **Chi square test; ***Fisher exact test*

DISCUSSIONS

Our study aimed to provide an overview of the health status in 345 women with ASCUS/ASC-H cytology after two years of follow-up. At enrolment, our results were consistent to the literature: ASCUS cytology was 5 times more common than ASC-H, but women with ASC-H had a significantly higher ATG2. HPV infection had high prevalence, similar in both groups and high-risk HPV types were found in similar proportion. HPV 16 was more common in ASC-H group, but without meeting the statistical significance. Histopathologic examination was far more common and with more severe lesions in ASC-H group.

The compliance of the women to medical recommendations was good, 86.4% of them attending the four planned visits, but HPV testing has been performed in a more reduced proportion, due to limited affordability. Cytology and colposcopy showed similar evolution in both groups, but the results should be interpreted with caution due to lost to or incomplete follow-up in 7% and 6.6% of cases respectively.

Histopathologic examination was needed in another one third of women during follow-up, but with no difference among groups. HPV testing showed results similar to enrolment, most common types being 16, 51, 31, 52 and 18 in ASCUS and 16, 31 and 18 in ASC-H, this time with significantly higher proportion of HPV 16 in the ASC-H group. Again, these results should be interpreted with caution, because on they are based last available testing (and not on last recommended one). The study limitations are related to characteristics of the subjects (selected population) and to limited availability of the HPV testing.

CONCLUSIONS

Despite the limitations, our study found more severity of colposcopic and histopathologic lesions in ASC-H women compared to ASCUS ones at enrolment, but a comparable clinical evolution after two years of follow-up. As regard of HPV infection, if testing available, it had high prevalence at enrolment, but also after two years of observation.

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