Liquid based cytology and HPV DNA testing in a Greek population compared to colposcopy and histology

S. Diamantopoulou[1], A. Spathis[2], A. Chranioti[2], D. Anninos[2], M. Stamataki[2], C. Chrelias[1], A. Pappas[1], I. Panayiotides[3], P. Karakitsos[2]

[1]Third Department of Obstetrics and Gynaecology, [2]Department of Cytopathology, [3]Second Department of Pathology, University General Hospital "ATTIKON", School of Medicine, National and Kapodistrian University of Athens, Athens (Greece)

Summary

Screening for cervical cancer in Greece is still unorganised and based on self- motivation. The purpose of this study was to evaluate the accuracy of cytological findings from a large observational population sample, originating from Western Athens, in association with reflex DNA test, colposcopic estimation, and final histologic diagnosis. The rate of invasive carcinoma, both squamous cell and adenocarcinoma, is indicative of a largely unscreened population. In this study, the estimated overall prevalence of human papilloma virus (HPV) was 41.1%, with HPV positivity at 37.4% of cytologically normal women. HPV testing did not seem to improve sensitivity of cytology for atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LGSIL) cases in identifying CIN 2+ lesions, but outperformed cytology in detecting CIN3+ for cytological high-grade squamous intraepithelial lesion (HGSIL) cases. For HGSIL cases sensitivity of colposcopy for detecting CIN3+ was comparable to cytology.

Key words: Cervix; Liquid based cytology; HPV DNA; Arrays; Colposcopy; Histology.

Introduction

Cervical cancer still remains a major health issue despite efforts made to reduce its incidence rates. Since 1999, human papilloma viruses (HPVs) are considered a necessary cause of invasive carcinoma development [1]. A significant problem in Greece is inadequate epidemiologic data of diseases such as cervical cancer, due to the fact that screening is opportunistic, based exclusively on self-motivation. Although Pap test is free of charge, the coverage rate of regular screening in urban areas is less than 30% [2]. Available data indicate that 550 new cases of cervical cancer per year occur in Greece [3]. Although vaccination for HPV has already been introduced in the national vaccination program, only 11% of the target population between 11 and 26 years of age has been vaccinated until now. Thus cervical cancer will, in all probability, remain a prevailing public health issue for the imminent future.

The purpose of the current study was to evaluate the accuracy of cytological findings from a large observational population sample in association with reflex DNA testing, colposcopic examination, and the final histologic diagnosis.

Materials and Methods

Study population

This is a cross-sectional study concerning 3,000 women with a median age of 34.3 ± 11.9 years (range 18 to 65 years), examined from March 2006 to September 2008. The population was consecutively recruited from the Third Department of Obstetrics and Gynaecology at the "Attikon" University Hospital.

The study population originated from Western Athens covering almost 0.1% of the capital's population of reproductive and post-menopausal aged women. All women proceeded voluntarily to the outpatient clinic for regular gynecological control and if they fulfilled the criteria of the protocol, they were enrolled in the study. Women with recent labor were excluded, while all participants signed an informed consent form. Research was performed with the approval of both the National and Kapodistrian University of Athens and the "Attikon" University Hospital Bioethics Committees.

Sample collection

Liquid based cytology (ThinPrep®) Pap tests were collected by means of a Broom's-like brush. The PreservCyt® vials were addressed to the Department of Cytopathology, for preparation of thin-layer slides using the ThinPrep 2000 Automated Slide Processor® according to the manufacturer's instructions.

Cytologic findings were interpreted according to the Bethesda classification system (TBS) into eight categories; NILM (negative for intraepithelial lesion or malignancy), ASC-US and ASC-H (atypical squamous cells of unknown significance or cannot exclude high SIL), LSIL and HSIL (low- or high-grade squamous intraepithelial lesion), SCC (squamous cell carcinoma) and AdenoCa (adenocarcinoma). ASCUS+ cytologic findings or positive HPV testing referred women for colposcopy; cervical biopsies were collected from colposcopical suspicious sites. In cases with no obvious abnormalities, at least three blind biopsies were taken. All women with indications consented to this procedure. Tissue fragments were fixed in a 10% buffered formalin solution and embedded into paraffin; four μm thick sections were stained with a standard haematoxylin/eosin (H&E) stain. The three-tiered cervical intraepithelial neoplasia (CIN) grading system was used for histological diagnosis.

Revised manuscript accepted for publication April 30, 2012

HPV DNA detection

ThinPrep® samples were stored at 4°C before DNA extraction. Procedures took place in two physically separated areas: the pre-PCR area, where samples were prepared and DNA was extracted and the post-PCR area, where products were amplified and visualised, minimizing the possibility of sample contamination with previously amplified products.

The commercially available kits Papillomavirus Clinical Arrays® (Genomica, Spain) and CLART® Human Papillomavirus 2 (Genomica, Spain) were used for HPV DNA extraction and genotyping. All samples were analysed for the presence of the following 35 HPV types which are divided, according to their oncogenic status, into two categories: low-risk: 6, 11,40, 42, 43, 44, 54,61,62,71,72,81, 83, 84 and 89 and high-risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82 and 85 [4].

HPV DNA was amplified using biotinylated PGMY primers that target a 450 bp long fragment of the viral L1 gene. Detection of the amplified PCR product was performed using a low-density microarray, anchored in a two ml array tube that allowed simultaneous detection of 35 different HPV types and included controls to ensure a feasible assay. Results' analysis was performed automatically.

Results

Cytology results and HPV detection

The study population originally included 3,000 women. 155 of these women (5.1%) were finally excluded due to lack of compliance or due to inadequate first sampling. Out of the final 2,845 samples, 2,442 had a negative (NILM) Pap smear (85.8%) whereas 403 (14.2%) were positive for cytological abnormalities of any kind according to TBS (Table 1). Among the 2,845 women included in the study population, HPV DNA test was positive in 1,234, i.e. an overall prevalence of the virus of 43.4%: HPV positivity was identified in 37.4% of NILM samples, 70.6% of ASCUS, 78.7% of LSIL samples, 100% of ASC-H, 93.8% of HSIL, 100% of AdenoCa, but only in 90% of the SCC.

Detection of genotypes among HPV positive women with NILM cytology identified a high-risk type in 81.5% of positive samples. Significantly, in 51.3% of these cases infection with multiple high-risk types was found, whereas 30.2% had a single high-risk type infection. The percentage of low-risk type infections (both single and multiple) was 18.5%.

Among HPV positive samples of ASCUS+ lesions, high-risk type infections were detected in 84.7% of ASCUS samples, 87.3% of LSIL samples, 95.6% of HSIL, 90% of AdenoCa, and 100% of ASC-H and SCC samples. The rate of multiple to single type infection was very close to 2:1 in all Bethesda categories, except for ASCUS (1:1) and SCC (1:2).

Colposcopical results

Among women with NILM cytological diagnosis, 914 tested positive for HPV and were therefore referred for colposcopy. Out of these, 867 (94.9%) had no colposcopic findings, whereas 31 (3.4%) had colposcopic findings compatible to LGSIL and only two (0.2%) were colposcopically evaluated as HGSIL. Colposcopy was inadequate in 14 cases. Results are summarized in Table 2.

All women with abnormal cytology were referred for colposcopy. From cases with LGSIL Pap test, 11.7% had an unremarkable colposcopy, 84.3% had colposcopic findings compatible with LGSIL, and 3.5% compatible with HGSIL. Women with HGSIL cytology had more characteristic findings at colposcopy and only 2.1% were found negative, whereas 70.7% had colposcopic findings compatible with as HSIL. Yet, 20.9% of cases with abnormal Pap test were colposcopically underestimated. Among women with ASCUS smears, 35.3% had no colposcopic indications of any abnormality, whereas 18.6% were estimated to have severe lesions. All women with ASC-H or SCC Pap test presented colposcopic findings. One case of AdenoCa was negative. Concerning cases

Table 1. — *ThinPrep diagnosis and biopsy results in relation with HPV DNA testing*.

HPV/ Cytology	Negative	Positive	High single	High multiple	Negative for high	Total
NILM	1,528	914 (37.4)	276 (30.2)	469 (51.3)	169 (18.5)	2442
ASC-US	30	72 (70.6)	23 (31.9)	38 (52.8)	11 (15.3)	102
LSIL	49	181 (78.7)	59 (32.6)	99 (54.7)	23 (12.7)	230
ASC-H	0	3 (100)	1 (33.3)	2 (66.7)	0	3
HSIL	3	45 (93.8)	13 (28.9)	30 (66.7)	2 (4.4)	48
SCC	1	9 (90)	7 (77.8)	2 (22.2)	0	10
AdenoCa	0	10 (100)	3 (30)	6 (60)	1 (10)	10
HPV/ Histology	Negative	Positive	High single	High multiple	Negative for high	Total
Normal	557 (62.4)	336 (37.6)	84 (25)	218 (64.9)	34 (10.1)	893
CIN 1	39 (12.1)	282 (87.9)	88 (31.2)	172 (61)	22 (7.8)	321
CIN 2	4 (6.6)	57 (93.4)	10 (17.5)	47 (82.5)	0	61
CIN 3	0	22 (100)	7 (31.8)	15 (68.2)	0	22
SCC	2 (15.4)	11 (84.6)	8 (72.7)	3 (27.3)	0	13
AdenoCa	0	7 (100)	5 (71.4)	2 (28.6)	0	7

Table 2. — *Correlation of cytology with colposcopic findings*.

Colposcopy/ Cytology	N	Negative	LSIL	HSIL	SCC	Inadequate
WNL	914	867 (94.9)	31 (3.4)	2 (0.2)	0	14 (1.5)
ASCUS	102	36 (35.3)	45 (44.1)	19 (18.6)	0	2 (2)
LSIL	230	27 (11.7)	194 (84.3)	8 (3.5)	0	1(0.5)
ASC-H	3	0	0	3 (100)	0	0
HSIL	48	1 (2.1)	9 (18.8)	34 (70.7)	3 (6.3)	1 (2.1)
SCC	10	0	0	2 (20)	8 (80)	0
AdenoCa	10	5 (50)	1 (10)	3 (30)	1 (10)	0
Total	1317	936	280	71	12	18

Table 3. — *Correlation of cytological and histological diagnoses*.

Colposcopy/ Cytology	N	Negative	CIN I	CIN II	CIN III	SCC	Adenoca
WNL	914	862 (94.3)	50 (5.5)	1 (0.1)	1 (0.1)	0	0
ASCUS	102	7 (6.9)	92 (90.2)	3 (2.9)	0	0	0
LSIL	230	23 (10)	173 (75.2)	30 (13)	3 (1.)	0	1 (0.4)
ASC-H	3	0	1 (33.3)	1 (33.3)	1 (33.3)	0	0
HSIL	48	1 (2.1)	5 (10.4)	26 (54.1)	14 (29.2)	2 (4.2)	0
SCC	10	0	0	0	0	10 (100)	0
AdenoCa	10	0	0	0	3(30)	1 (10)	6 (60)
Total	1317	893	321	61	22	13	7

Table 4. — *Sensitivity, specificity, positive, and negative predictive values of the screening tests.*

	Sensitivity for ≥ CIN 2	Specificity for ≥ CIN 2	PPV for ≥ CIN 2	NPV for ≥ CIN 2	Sensitivity for ≥ CIN 3	Specificity for ≥ CIN 3	PPV for ≥ CIN 3	NPV for ≥ CIN 3
ThinPrep Pap (≥ ASCUS)	98.06%	75.12%	25.06%	99.78%	97.62%	71.61%	10.17%	99.89%
ThinPrep Pap (≥ LGSIL)	95.15%	83.28%	32.56%	99.51%	97.62%	79.61%	13.62%	99.90%
ThinPrep Pap (≥ ASC-H)	62.14%	99.42%	90.14%	96.87%	88.10%	97.33%	52.11%	99.60%
ThinPrep Pap (≥ HSIL)	60.19%	99.51%	91.18%	96.72%	85.71%	97.49%	52.94%	99.52%
CLART HPV Positive	94.17%	49.09%	13.57%	99.00%	95.24%	47.06%	5.95%	99.67%
Colposcopy (any abnormal)	71.29%	75.71%	19.83%	96.90%	82.50%	73.79%	9.09%	99.25%

with cytologic diagnosis of AdenoCa, 83.3% had colposcopic findings compatible with LGSIL or HGSIL.

It is noteworthy that 18.8% of cytologically HSIL women were colposcopied as of lower significance abnormality and even one case as negative (2.1%). Almost one-third of high-grade lesions were not identified during colposcopy, although performed by an expert clinician.

Histological results

Comparison of cytologic and histological results is presented at Table 3. Histologically-normal were 94.3% of the cytology NILM HPV-positive cases, 10% of the LSIL, 2.1% of the HSIL, and 6.9% of the ASCUS cases. None of the ASC-H, AIS, SCC, and AdenoCa were histological normal. Among cytological NILM samples, 5.5% had a biopsy diagnosis of CIN 1; one case was histologically diagnosed as CIN 2, and one as CIN 3.

Cytological LSIL cases had an underlying lesion of CIN2+ in 14.7% of cases, including one case of AdenoCa. The cytological diagnosis of HSIL was more efficient, when compared to the golden standard of histology, since there was only 12.5% of ≤ CIN 1. In ASCUS cases, 90.2% had a ≤ CIN 1 in biopsy. While concordance was found in 60% of AdenonoCa, no case was lost, as the remaining were CIN3+.

Only one case (2.1%) of HSIL was histologically normal and five (10.4%) were CIN 1, forming the total percentage of those with lighter or no abnormality demonstrated by histology at 12.5%. The one HSIL case with normal histological diagnosis was the same one that was colposcopically negative.

Correlation of histology with HPV testing results revealed that 62.4% of the diagnosed as NILM samples were actually HPV-positive. In particular 89.9% had high-risk type infection, with an overbalance of multiple types versus single type infection. This phenomenon was identified also in all CIN lesions. While 100% of the tested samples with verified AdenoCa were HPV positive, especially with a high-risk single type infection, only 84.6% of the SCC was positive and with the same characteristics: high-risk single type infection.

The correlation of histology with colposcopy revealed an underestimation of the biopsy confirmed CIN 2 cases at 54.1%. There was a concordance of CIN2+ histology and high-grade lesion colposcopically at 53%. Since there was a subsuming of all "suspicious HPV" histological diagnoses at the HPV category, as already mentioned, there were 22.2% of cases estimated as negative colposcopically. All tests' performance is summarized in Table 4 compared to the golden standard of histology.

Discussion

In this study accuracy of cytological findings were evaluated by comparing cytological diagnoses along with reflex DNA testing, colposcopic examination, and final histologic diagnosis from samples obtained according to protocol. In the present study, the authors estimated the overall prevalence of HPV at 41.1%. Other studies in the Greek population demonstrated prevalence ranging from 2.5% up to 60% [2, 5-8] while in a more recent study, HPV was detected by consensus PCR in 31.3% of the samples [9]. As new typing methods increase the number of HPV types detected, it is prospective that more infections will be identified and the prevalence will augment.

HPV DNA was positive in 37.4% of cytologically normal women in agreement with some studies [6, 10]; yet, rates of HPV detection in such cases vary widely in literature ranging from 3% to 34.3% when consensus PCR had been used [11-15] and probably reflected the high analytical sensitivity of the detection method used. Although almost 80% of women have transient infections [16], since HPV infection precedes the development of SILs [17], women with normal cytology but HPV positive should be prospectively followed by their gynecologist and submitted to cytology and other testing where appropriate [18, 19]. In the vast majority of women with normal cytology, who were referred to colposcopy, no detectable lesion was identified; further supporting that HPV DNA testing cannot be used in screening due to its low PPV (Table 4). Cases histologically confirmed as CIN 1 but negative for HPV DNA could be the result of either viral clearance during the time window between cytology and histology, poor sampling during cytology testing, or loss if the L1 viral gene that is the target of the

molecular technique used at the present study, due to viral integration into the host genome [1]. HPV DNA negative squamous cell carcinomas may, also be related to full integration of HPV or to a "passenger effect" of the virus, where viral replication was inhibited in that specific genetic environment [20].

The reported results (79.4% HPV-DNA positive among cytologically abnormal samples) confirm the causal relationship between HPV infection and abnormal cytology, in concordance with most studies published so far, demonstrating an increase of HPV prevalence related to higher grading of squamous intraepithelial lesions [11, 21-24]. In the study population, 3.4% was diagnosed as ASC-US and 0.1% as ASC-H plus. ASC-H is reported in the literature in 0.27 - 0.6% of all Pap test results [25-27] while the mean frequency of detection of ASC-US in the USA is 4.7% of all smears [28]. The results in the present study seem to be in concordance with the literature estimating that the frequency of ASC-US should not exceed two to three times the frequency of LSIL [25, 29-35]. The authors must also mention that the number of ASC-US cases is significantly low for the "high-risk" study population evaluated in the current study, where cervical cancer incidence was 0.7%, rather than the 0.1% anticipated in the general population. Approximately 70% of the ASC-US smears and all 100% of the ASC-H were HPV positive. Colposcopy verified the presence of abnormalities in 62.7% and 100% respectively as did histology in 93.1% of ASCUS and 100% of ASC-H cases. Since sampling collection was performed by experienced gynecologists and examined by trained cytopathologists with at least five years experience in liquid based cytology (LBC), these results were more or less as anticipated. Yet, histologic results raised questions about screening intervals for these patients and about treatment options, since according to a meta-analysis, the absolute risk of underlying CIN2+ and CIN3+ among women with ASCUS is on average 9-10% and 4-5% respectively [36]. The ALTS study documented a cumulative risk of high-grade disease at 26.7% for women with HPV-positive ASCUS [37].

The use of HPV testing has been recommended for women with ASC-US [38]. HPV DNA testing seems to be more sensitive than colposcopy in ASCUS cases [39]. In this study, HPV testing did not seem to improve sensitivity of cytology in ASCUS cases in identifying severe lesions (Table 4). Keeping in mind that, although the HPV assay results were performed as quicky as possible, yet the interval of time may have altered the virus status and that may have affected the test performance. The ASCCP and ACOG management guidelines for women with HPV-positive ASC-US recommend immediate referral to colposcopy [21-22, 29, 40]. Literature reports [21-22, 39, 41-42] that 20-60% of ASCUS cases are associated with CIN colposcopic diagnosis, yet among them, 70% are CIN 1. The results in this study agree with the literature, since in 62.7% of all ASCUS cases the presence of a lesion was colposcopically identified. However, the results showed that for such cases colposcopy did not seem to improve sensitivity of cytology in identifying CIN2 and CIN3 lesions with specificity. For women with cytological ASC-H diagnosis, the association with high-risk HPV was suggested to carry a higher risk of CIN 2+ in 40% [43], yet in 66% in this study. The detection of HPV types among women with ASC-H diagnosis seems to improve sensitivity of cytology for detecting both CIN2 and CIN3 cases. This clue, along with that colposcopy, also seems to be a more sensitive method, and must be taken into account in order to manage women with ASC-H cytology.

In cytological LSIL cases, neither HPV testing, nor colposcopy outperformed cytology that had comparable, if not better results. The pooled results of a recent meta-analysis indicated that reflex HPV testing is insufficiently discriminative in case of LSIL, as the large majority of LSIL cases were high-risk HPV positive [44]. Yet, the cumulative risks of CIN2+ and CIN3+ among HPV positive women with LSIL cytology resulted in 30.3% and 17.2% respectively according to the recent TOMBOLA study [45]. These women were set to a more extensive follow-up during second and third round of this study, by protocol. The 14.7% of LGSIL cases that actually had an underlying CIN 2+ lesion during the first round of this study, is estimated to decrease at 12 months and even more at 24 months [46, 47]. It must be noted that for some cases there was a significant time delay between cytology and referral to colposcopy, and some lesions may have regressed.

Sensitivity of HPV testing for detecting CIN3+ for cytological HSIL cases outperformed cytology, yet with significantly lower specificity. When the HSIL+ cytological lesion cutoff point was used, sensitivity of colposcopy for detecting CIN3+ was comparable to cytology, as shown by others [48].

The rate of invasive carcinoma discovered in this study is indicative of a largely unscreened population. Among cytologically SCC samples, 77.8% had single high-risk type infections. This fact seems to be in concordance with the hypothesis that a certain type may become dominant over others as the disease progresses [49] and that cervical neoplasia is a result of clonal expansion of a cell infected with a single type HPV [50]. Two out of 13 SCC histologically-verified cases tested negative for HPV. For these cases either cervical cancer may have been caused by a different mechanism, or an HPV type not detected by the methods used, or the HPV causing the cervical carcinogenesis may have been lost, since some carcinogenic types may have presented only as passengers [51].

A cytological result of adenocarcinoma in situ (AIS), as demonstrated by several studies, is associated with 48-69% risk of biopsy-confirmed AIS and 38% risk of invasive adenocarcinoma. In the presented study, there seems to be an agreement with these findings, although the small number of cases should be kept in mind. All of the cases tested HPV positive, yet the 2001 Consensus Conference concluded that there was insufficient data to allow an assessment of the role of HPV DNA testing in the management of AGC and AIS [52]. The results indi-

cated also the anticipated: for such cases, colposcopy is rather difficult to set a diagnosis.

The overall results, as presented in Table 4, coincide with TBS 2001 recommendations, and indicate that when cytological diagnosis stands on a very good level, immediate referral to colposcopy is acceptable. Given the extremely high-negative predictive value of Pap test, the actual possibility of a CIN 2+ lesion underlying a NILM diagnosis is quite small. If the cut-off cytological diagnosis for referral is set at LGSIL+ a significant gain in specificity with a minor drop of sensitivity is observed, compared to ASC-US+ with an end-point of CIN 2+. On the other hand, by setting the ASC-H+ diagnosis as the cut-off, a great gain in PPV, NPV and specificity is observed, while sensitivity is cut down. Combining results from Table 4 indicate that with an ASC-H+ diagnosis and a positive HPV DNA test, colposcopists must be extremely careful because there is a great possibility of an underlying severe lesion. On the other hand, a NILM cytology combined with a negative colposcopy, has an underlying lesion in only 0.8%. Since in almost 40% of such cases HPV DNA test is positive, this particular examination is not cost-effective.

The limitation of a population that is not systematically screened, but is consecutively enrolled is of course recognized. Despite the excellent results of cytology in this study, it is well-known that screening for cytological changes may have limited sensitivity and findings are not always reproducible [51]. In Greece the financial value of colposcopy is lower than HPV testing. Although sensitivity and specificity of colposcopy are moderate, negative predictive value is exceptionally good. From both the clinician and patient perspectives, the predictive values are the most important parameters. Positive predictive value is accepted to be very low, since the visual changes caused by HPV and identified by colposcopy are quite common. Thus, the high-negative predictive value reassures that women tested negative could be examined periodically by test Pap and colposcopy with larger yet safer time intervals [53]. Moreover, molecular HPV testing should not be introduced without careful planning; results of such testing should be communicated and explained appropriately in the context of prevalence of the disease.

Acknowledgments

This project was funded by a research grant from the Greek Ministry of Development-GSRT, Project AKAKOS (code ATT.95).

References

[1] Walboomers J.M., Jacobs M.V., Manos M.M., Bosch F.X., Kummer J.A., Shah K.V. *et al*.: "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide". *J. Pathol*., 1999, *189,* 12.

[2] Agorastos T., Lambropoulos A.F., Sotiriadis A., Mikos T., Togaridou E., Emmanouilides C.J.: "Prevalence and distribution of high-risk human papillomavirus in Greece". *Eur. J. Cancer Prev*., 2009, *18*, 504.

[3] Cancer W.I.I.C.o.H.a.C., Human Papillomavirus and Related Cancers in Greece, 2010.

[4] Dunne E.F., Unger E.R., Sternberg M., McQuillan G., Swan D.C., Patel S.S. *et al*.: "Prevalence of HPV infection among females in the United States". *JAMA,* 2007, *297,* 813.

[5] Agorastos T., Dinas K., Lloveras B., Bosch F.X., Kornegay J.R., Bontis J.N. *et al*.: "Cervical human papillomavirus infection in women attending gynaecological outpatient clinics in northern Greece". *Eur. J. Cancer Prev*., 2004, *13*, 145.

[6] Kroupis C., Thomopoulou G., Papathomas T.G., Vourlidis N., Lazaris A.C.: "Population-based study of human papillomavirus infection and cervical neoplasia in Athens, Greece". *Epidemiol. Infect*., 2007, *135*, 943.

[7] Panotopoulou E., Tserkezoglou A., Kouvousi M., Tsiaousi I., Chatzieleftheriou G., Daskalopoulou D. *et al*.: "Prevalence of human papillomavirus types 6, 11, 16, 18, 31, and 33 in a cohort of Greek women". *J. Med. Virol*., 2007, *79*, 1898.

[8] Stamataki P., Papazafiropoulou A., Elefsiniotis I., Giannakopoulou M., Brokalaki H., Apostolopoulou E. *et al*.: "Prevalence of HPV infection among Greek women attending a gynecological outpatient clinic". *BMC Infect. Dis*., 2010, *10,* 27.

[9] Tsiodras S., Georgoulakis J., Chranioti A., Voulgaris Z., Psyrri A., Tsivilika A. *et al*.: "Hybrid capture vs. PCR screening of cervical human papilloma virus infections. Cytological and histological associations in 1270 women". *BMC Cancer*, 2010, *10*, 53.

[10] Stoler M.H., Schiffman M.: "Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study". *JAMA,* 2001, *285*, 1500.

[11] Nobbenhuis M.A., Walboomers J.M., Helmerhorst T.J., Rozendaal L., Remmink A.J., Risse E.K. *et al*.: "Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study". *Lancet,* 1999, *354*, 20.

[12] Kulmala S.M., Syrjanen S., Shabalova I., Petrovichev N., Kozachenko V., Podistov J. *et al*.: "Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools". *J. Clin. Microbiol*., 2004, *42,* 2470.

[13] Yamazaki H., Sasagawa T., Basha W., Segawa T., Inoue M.: "Hybrid capture-II and LCR-E7 PCR assays for HPV typing in cervical cytologic samples". *Int. J. Cancer,* 2001, *94,* 222.

[14] Cuzick J., Beverley E., Ho L., Terry G., Sapper H., Mielzynska I. *et al*.: "HPV testing in primary screening of older women". *Br. J. Cancer,* 1999, *81*, 554.

[15] Riethmuller D., Gay C., Bertrand X., Bettinger D., Schaal J.P., Carbillet J.P. *et al*.: "Genital human papillomavirus infection among women recruited for routine cervical cancer screening or for colposcopy determined by Hybrid Capture II and polymerase chain reaction". *Diagn. Mol. Pathol*., 1999, *8,* 157.

[16] Meijer C.J., Snijders P.J., van den Brule A.J.: "Screening for cervical cancer: should we test for infection with high-risk HPV?". *CMAJ*, 2000, *163,* 535.

[17] Winer R.L., Kiviat N.B., Hughes J.P., Adam D.E., Lee S.K., Kuypers J.M. *et al*.: "Development and duration of human papillomavirus lesions, after initial infection". *J. Infect. Dis*., 2005, *191*, 731.

[18] Richart R.M., Masood S., Syrjanen K.J., Vassilakos P., Kaufman R.H., Meisels A. *et al*.: "Human papillomavirus". International Academy of Cytology Task Force summary. Diagnostic Cytology Towards the 21st Century: An International Expert Conference and Tutorial. *Acta Cytol*., 1998, *42,* 50.

[19] Lorincz A.T., Richart R.M.: "Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs". *Arch. Pathol. Lab. Med*., 2003, *127,* 959.

[20] Kraus I., Molden T., Erno L.E., Skomedal H., Karlsen F., Hagmar B.: "Human papillomavirus oncogenic expression in the dysplastic portio; an investigation of biopsies from 190 cervical cones". *Br. J. Cancer*, 2004, *90*, 1407.

[21] Cox J.T., Lorincz A.T., Schiffman M.H., Sherman M.E., Cullen A., Kurman R.J.: "Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance". *Am. J. Obstet. Gynecol*., 1995, *172*, 946.

[22] Manos M.M., Kinney W.K., Hurley L.B., Sherman M.E., Shieh-Ngai J., Kurman R.J. *et al*.: "Identifying women with cervical neoplasia: using human papillomavirus DNA testing for equivocal Papanicolaou results". *JAMA,* 1999, *281*, 1605.

[23] Meijer C.J., van den Brule A.J., Snijders P.J., Helmerhorst T., Kenemans P., Walboomers J.M.: "Detection of human papillomavirus in cervical scrapes by the polymerase chain reaction in relation to cytology: possible implications for cervical cancer screening". *IARC Sci. Publ*., 1992, 271.

[24] Clavel C., Masure M., Bory J.P., Putaud I., Mangeonjean C., Lorenzato M. *et al*.: "Hybrid Capture II-based human papillomavirus detection, a sensitive test to detect in routine high-grade cervical lesions: a preliminary study on 1518 women". *Br. J. Cancer,* 1999, *80,* 1306.

[25] Cox J.T.: "Management of women with cervical cytology interpreted as ASC-US or as ASC-H". *Clin. Obstet. Gynecol*., 2005, *48,* 160.

[26] Alli P.M., Ali S.Z.: "Atypical squamous cells of undetermined significance-rule out high-grade squamous intraepithelial lesion: cytopathologic characteristics and clinical correlates". *Diagn. Cytopathol*., 2003, *28*, 308.

[27] Simsir A., Ioffe O., Sun P., Elgert P., Cangiarella J., Levine P.H.: "Effect of Bethesda 2001 on reporting of atypical squamous cells (ASC) with special emphasis on atypical squamous cells-cannot rule out high grade (ASC-H)". *Diagn. Cytopathol*., 2006, *34*, 62.

[28] Davey D.D., Neal M.H., Wilbur D.C., Colgan T.J., Styer P.E., Mody D.R.: "Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology". *Arch. Pathol. Lab. Med*., 2004, *128*, 1224.

[29] Solomon D., Schiffman M., Tarone R.: "Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial". *J. Natl. Cancer Inst*., 2001, *93,* 293.

[30] Sodhani P., Gupta S., Singh V., Sehgal A., Mitra A.B.: "Eliminating the diagnosis atypical squamous cells of undetermined significance: impact on the accuracy of the Papanicolaou test". *Acta Cytol*., 2004, *48*, 783.

[31] Yarandi F., Izadi Mood N., Mirashrafi F., Eftekhar Z.: "Colposcopic and histologic findings in women with a cytologic diagnosis of atypical squamous cells of undetermined significance". *Aust. N Z J Obstet. Gynaecol*., 2004, *44,* 514.

[32] Davey D.D., Naryshkin S., Nielsen M.L., Kline T.S.: "Atypical squamous cells of undetermined significance: interlaboratory comparison and quality assurance monitors". *Diagn. Cytopathol*., 1994, *11*, 390.

[33] Gerber S., De Grandi P., Petignat P., Mihaescu A., Delaloye J.F.: "Colposcopic evaluation after a repeat atypical squamous cells of undetermined significance (ASCUS) smear". *Int. J. Gynaecol. Obstet*., 2001, *75*, 251.

[34] Lee S.J., Jung K.L., Lee J.W., Song S.Y., Kim B.G., Lee J.H. *et al*.: "Analyses of atypical squamous cells refined by the 2001 Bethesda System: the distribution and clinical significance of follow-up management". *Int. J. Gynecol. Cancer,* 2006, *16,* 664.

[35] Juskevicius R., Zou K.H., Cibas E.S.: "An analysis of factors that influence the ASCUS/SIL ratio of pathologists". *Am. J. Clin. Pathol*., 2001, *116*, 331.

[36] Arbyn M., Sasieni P., Meijer C.J., Clavel C., Koliopoulos G., Dillner J.: "Clinical applications of HPV testing: a summary of meta-analyses". *Vaccine,* 2006, *24* (suppl. 3), S3/78.

[37] Cox J.T., Schiffman M., Solomon D.: "Prospective follow-up suggests similar risk of subsequent cervical intraepithelial neoplasia grade 2 or 3 among women with cervical intraepithelial neoplasia grade 1 or negative colposcopy and directed biopsy". *Am. J. Obstet. Gynecol*., 2003, *188,* 1406.

[38] Kaufman R.H., Adam E., Icenogle J., Reeves W.C.: "Human papillomavirus testing as triage for atypical squamous cells of undetermined significance and low-grade squamous intraepithelial lesions: sensitivity, specificity, and cost-effectiveness". *Am. J. Obstet. Gynecol*., 1997, *177*, 930.

[39] Bergeron C., D. Jeannel, J. Poveda, P. Cassonnet, G. Orth: "Human papillomavirus testing in women with mild cytologic atypia". *Obstet. Gynecol.,* 2000, *95,* 821.

[40] ASCUS-LSIL Triage Study (ALTS) Group, *et al*.: "Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance". *Am. J. Obstet. Gynecol*., 2003, *188*, 1383.

[41] Ferris D.G., T.C. Wright Jr., M.S. Litaker, R.M. Richart, A.T. Lorincz, X.W. Sun *et al*.: "Comparison of two tests for detecting carcinogenic HPV in women with Papanicolaou smear reports of ASCUS and LSIL". *J. Fam. Pract,* 1998, *46,* 136.

[42] Wright T.C. Jr., A. Lorincz, D.G. Ferris, R.M. Richart, A. Ferenczy, I. Mielzynska *et al*.: "Reflex human papillomavirus deoxyribonucleic acid testing in women with abnormal Papanicolaou smears". *Am. J. Obstet. Gynecol.,* 1998, *178*, 962.

[43] Sherman M.E., D. Solomon, M. Schiffman: "Qualification of ASCUS. A comparison of equivocal LSIL and equivocal HSIL cervical cytology in the ASCUS LSIL Triage Study". *Am. J. Clin. Pathol*., 2001, *116,* 386.

[44] Arbyn M., P. Martin-Hirsch, F. Buntinx, M. Van Ranst, E. Paraskevaidis, J. Dillner: "Triage of women with equivocal or low-grade cervical cytology results: a meta-analysis of the HPV test positivity rate". *J. Cell. Mol. Med.,* 2009, *13,* 648.

[45] Arbyn M., P. Martin-Hirsch, N. Wentzensen: "Human papillomavirus- based triage of women showing a cervical cytology result of borderline or mild dyskaryosis". *BJOG,* 2010, *117*, 641.

[46] Moscicki A.B., M. Schiffman, S. Kjaer, L.L. Villa: "Updating the natural history of HPV and anogenital cancer". *Vaccine,* 2006, *24* (suppl. 3), S3/42.

[47] Ho G.Y., R. Bierman, L. Beardsley, C.J. Chang, R.D. Burk: "Natural history of cervicovaginal papillomavirus infection in young women". *N. Engl. J. Med*., 1998, *338*, 423.

[48] Mitchell M.F., D. Schottenfeld, G. Tortolero-Luna, S.B. Cantor, R. Richards-Kortum: "Colposcopy for the diagnosis of squamous intraepithelial lesions: a meta-analysis". *Obstet. Gynecol*., 1998, *91,* 626.

[49] Hwang H.S., M. Park, S.Y. Lee, K.H. Kwon, M.G. Pang: "Distribution and prevalence of human papillomavirus genotypes in routine pap smear of 2,470 Korean women determined by DNA chip". *Cancer Epidemiol. Biomarkers Prev*., 2004, *13,* 2153.

[50] Ho G.Y., A.S. Kadish, R.D. Burk, J. Basu, P.R. Palan, M. Mikhail *et al*.: "HPV16 and cigarette smoking as risk factors for high-grade cervical intra-epithelial neoplasia". *Int. J. Cancer,* 1998, *78,* 281.

[51] Wright T.C. Jr., J.T. Cox, L.S. Massad, J. Carlson, L.B. Twiggs, E.J. Wilkinson: "2001 consensus guidelines for the management of women with cervical intraepithelial neoplasia". *Am. J. Obstet. Gynecol*., 2003, *189*, 295.

[52] Sahebali S., C.E. Depuydt, G.A. Boulet, M. Arbyn, L.M. Moeneclaey, A.J. Vereecken *et al*.: "Immunocytochemistry in liquid-based cervical cytology: analysis of clinical use following a cross-sectional study". *Int. J. Cancer,* 2006, *118,* 1254.

[53] Ferreccio C., M.C. Bratti, M.E. Sherman, R. Herrero, S. Wacholder, A. Hildesheim *et al*.: "A comparison of single and combined visual, cytologic, and virologic tests as screening strategies in a region at high risk of cervical cancer". *Cancer Epidemiol. Biomarkers Prev*., 2003, *12*, 815.

Address reprint requests to:
P. KARAKITSOS, M.D.
Department of Cytopathology
Univ. General Hospital "Attikon"
Rimini Str. 1
12462 Chaidari, Athens (Greece)
e-mail: pkaraki@med.uoa.gr