

Multicentric assessment of cervical HPV infection co-factors in a large cohort of Greek women

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Summary

Purpose of Investigation: Despite the general principle that persistent high risk human papillomavirus (hr-HPV) infection may progressively cause cervical cancer (CxCa), demographic aspects may still identify groups at high risk for HPV infection and consequently for precancerous lesions. The role of demographic parameters on cervical status, the diagnostic accuracy of cytology, HPV genotyping, and their possible combinations, were investigated in this study. **Materials and Methods:** 11,072 women from diverse locations across Greece participated in the study. Liquid Based Cytologic (LBC) assessment was followed, when necessary, by HPV-genotyping and histologic confirmation. Demographic characteristics were also assessed. **Results:** Life style parameters such as age, number of sexual partners, condom use, parity and marital status, education level, and combination of smoking/condom use, were significant factors for CIN2+ lesions ($p < 0.05$). For LSIL+ detection, cytology had maximum sensitivity: 96.90%, HPV-typing: 72.55% and co-testing: 98.97%, for HSIL+ detection: 98.49%, 92.22%, and 99.55% respectively. **Conclusions:** There are notable relationships between lifestyle, demographic details, and cervical status. Despite the differences in sensitivity and specificity levels, co-testing might offer some marginal improvement in the detection of particular pre-cancerous conditions.

Key words: Cervical cancer; Biomarkers; Cervical cytology; Cervical intraepithelial neoplasia; HPV.

Introduction

Cervical cancer (CxCa) is one of the third most common cancers in women, and ranks as the fourth neoplastic cause of women death worldwide [1]. The majority of the cases and associated deaths are observed in developing countries. This is primarily caused due to lack of screening programs, which aim to detect early precancerous lesions where treatment has a positive impact in outcome. Despite the benefits of established cervical cancer screening, this particular malignancy remains a serious problem in the developed countries as well.

Cervical cancer and high-risk human papillomavirus (hr-HPV) infection have a causative relation; moreover HPV appears to be the most common sexually transmitted infection worldwide. Despite the high rates of viral regression at various stages, a persistent infection may lead to cervical precancer and eventually to cancer. More than 100 different HPV subtypes have been identified, however among them, only 15 are considered oncogenic and may lead to invasive disease. Several molecular HPV related tests within the portfolio of CxCa prevention have been developed. These tests are either used as complimentary tests to the liquid

Table 1. — *Histological status of the cases.*

NAME OF CATEGORY	DESCRIPTION	No of cases	% of cases		
CLINICALLY NEGATIVE	Cyto: (-)ve	2329	35.7		
	Colpo: (-)ve				
	HPV: DNA (-)ve				
	Histology unavailable				
	Cyto: (-)ve			15	0.2
	Colpo: AWE				
	HPV DNA: (-)ve				
CLINICALLY LSIL	Histology unavailable	860	13.2		
	Cyto: (-)ve				
	Colpo: (-)ve				
	HPV DNA: (+)ve (any subtype)				
	Histology unavailable				
	Cyto: ASCUS or LSIL			522	8
	Colpo: low grade lesion (HPV or CIN1 or LSIL)				
	HPV DNA: not examined				
	Histology unavailable				
	Cyto: ASC-H or HSIL or AGC or AGUS			63	1
	Colpo: normal or low grade lesion (HPV or CIN1 or LSIL)				
	HPV DNA: hr (-)ve				
	Histology unavailable				
	Colpo: AWE			72	1.1
HPV DNA: available, not negative					
Histology unavailable					

Table 2. — *Correlation matrix of cytology histology, including CLINICALLY NEGATIVE and LSIL cases.*

Cytology	Histology							Grand total
	NEGATIVE	CN*	LSIL	CLINICALLY LSIL	HSIL	SCC	ADENO-CA	
INADEQUATE	12		27	5	15	2		61
NEGATIVE	103	2329	98	10	9	1		2550
LSIL	122		552	1676	116		1	2467
ASCUS	71		190	527	34			822
HSIL	21		68	5	424	11	2	531
ASC-H	11		9	7	21	2		50
AGC	1						1	2
AGUS	1				1			2
SCC	1				1	21		25
ADENO-CA	1					1	11	13
CA						4		4
Grand total	344	2329	944	2230	621	42	17	6527

* CN: clinically negative.

based Papanicolaou test (“co-testing”) or as standalone and competing tests [2-6].

Nowadays, there are numerous HPV based tests, which are linked to oncogenic activation, predominantly HPV DNA identification and typing [6-15], as well as mRNA identification of the viral E6/E7 oncogenes. Among these, mRNA typing with nucleic acid based amplification (NASBA) [16-18] and mRNA-Flow-FISH techniques [18-20] have produced promising results in screening programs with increased positive predictive value (PPV) and reduction of unnecessary referrals or recalls to colposcopy [21-

27]. Furthermore, immunocytochemical detection of p16 has augmented the diagnostic accuracy of the Papanicolaou smear [7, 28-33], while methylation markers [34-36] and micro RNAs [37, 38], when applied as ancillary tests, have shown promising results.

Among the plethora of published studies, besides those attempting to clarify the role of each specific HPV-related biomarker, there is a variety of trials aiming to model the impact of molecular techniques as alternatives to the Pap smear, in the context of secondary cervical screening option or triage methodology [16, 21-24, 39-48]. However,

Table 3. — Studied demographic characteristics for the non-CIN2 group and CIN2+ group along with statistical significance.

Demographic characteristic	Total # of cases	Non-CIN2+ n	Percentage or mean value \pm SD	CIN2+ n	Percentage or mean value \pm SD	<i>p</i> *
Age	5439	4870	35.53 \pm 11.39	569	36.65 \pm 9.84	< 0.05
Age of first sexual intercourse	3470	2980	18.58 \pm 2.68	490	17.99 \pm 2.27	< 0.0001
Number of sexual partners	3344	2867	4.34 \pm 5.04	477	6.17 \pm 7.78	< 0.0001
Condom use (yes)	517	413	42.86%	114	42,1%	0.0566
Parity	675	508	1.61 \pm 2.16	167	1.57 \pm 1.81	0.75
Smoking (yes)	1896	1536	64.91%	360	69.44%	0.1167
Marital status = non married	505	382	45.22%	123	32.52%	0.0175
Marital status = married	505	382	43.41%	123	52.84%	0.0851
Marital status = divorced	505	382	9.04%	123	13.01%	0.2699
Education = university	436	333	65.76%	103	52.42%	0.0199
Education = basic	436	333	31.24%	103	38.84%	0.1891
Education = below basic	436	333	3.00%	103	8.74%	0.0266
Smoking and no condom use	1004	768	27.34%	236	42.37%	0.0001

* *Bold entries indicate statistically significant differences. t - test*

Table 4. — Correlation matrix between cytology and histology

Histology	Cytological outcome		Total
	NEGATIVE (i.e. NORMAL)	POSITIVE (i.e. ASCUS+)	
NEGATIVE or CIN	2432	229	2661
LSIL or CLIN. LSIL	108	3034	3142
HSIL	9	597	606
CxCa	1	56	57
Total	2550	3916	6466

Table 5. — Confusion matrix between co-testing and histology.

Histology	Cytological outcome		Total
	NEGATIVE (i.e. NORMAL Papanicolaou test AND negative HPV DNA typing)	POSITIVE (i.e. ASCUS+ in cytology or any HPV type present)	
NEGATIVE or CIN	1146	1128	2274
LSIL or CLIN. LSIL	36	3091	3127
HSIL	3	603	606
CxCa		56	56
Total	1185	4878	6063

the performance of these studies has shown significant diversity, depending mainly on the disease incidence, prevalence of HPV infection, and specific characteristics of each study population [49-52].

Based on a large multi-centric database of women attending different affiliated University Hospital colposcopy clinics, and focusing on the initial visit of each woman, we have conducted a study aiming to: a) identify and evaluate the role of individual “life-style” profile as an indicator of harboring cervical intraepithelial neoplasia (CIN) and: b) assess the relevant performance of combined cytology and several molecular HPV markers in the detection of low and high-grade squamous intraepithelial lesions (LSIL and HSIL), as well as the performance of HPV DNA test as a

standalone triage test.

Materials and Methods

This multi-centric retrospective observational study was conducted with the participation of several institutions across Greece: a) the Second Department of Pathology, b) the Department of Cytopathology and c) the 3rd Department of Obstetrics and Gynecology of the Medical School of Athens, d) the Department of Obstetrics and Gynecology of University Hospital of Ioannina, e) the Unit of Molecular Cytopathology and f) the Department of Obstetrics and Gynecology of University Hospital of Thessaloniki, and g) the Biomedical Engineering Laboratory, of the National Technical University of Athens. The study was conducted up

Table 6. — Performance of cytology, HPV typing alone, and co-testing via various combinations for the detection of cervical abnormalities (cytological cut-off: ASCUS+, LSIL+ and ASC-H+, HPV DNA typing cut-offs: Any HPV type, Any HR type and types 16/18). PPV: positive predictive value, NPV: negative predictive value, FPR: false positive rate, FNR: false negative rate, OA: overall accuracy, PLR: positive likelihood ratio, NLR: negative likelihood ratio, odds ratio: (specificity x sensitivity)/[(1-specificity) x (1-sensitivity)].

Histological cut off	Triage method	Sensitivity	Specificity	PPV	NPV	FPR	FNR	OA	PLR	NLR	Odds ratio	Youden Index
Histology LSIL +	Cytology ASCUS+	96.90%	91.39%	94.15%	95.37%	8.61%	3.10%	94.63%	11.26	0.03	331.83	88.29%
	Cytology LSIL+	77.16%	94.06%	94.89%	74.23%	5.94%	22.84%	84.12%	13	0.24	53.52	71.22%
	Cytology ASC-H+	15.53%	98.65%	94.26%	44.96%	1.35%	84.47%	49.74%	11.48	0.86	13.41	14.18%
	HPV any type	72.55%	54.29%	70.83%	56.39%	45.71%	27.45%	65.33%	1.59	0.51	3.14	26.84%
	HPV HR positive	64.68%	60.22%	71.32%	52.71%	39.78%	35.32%	62.92%	1.63	0.59	2.77	24.90%
	HPV 16,18 positive	24.76%	89.25%	77.89%	43.68%	10.75%	75.24%	50.25%	2.3	0.84	2.73	14.01%
	Co-testing (cytology ASCUS + & HPV typing positive)	98.97%	50.40%	76.88%	96.71%	49.60%	1.03%	80.75%	2	0.02	97.69	49.37%
	Co-testing (cytology ASCUS + & HPV HR positive)	98.50%	56.39%	77.37%	96.13%	43.61%	1.50%	81.75%	2.26	0.03	84.92	54.89%
	Co-testing (cytology ASCUS + & HPV 16,18 positive)	97.47%	82.02%	89.14%	95.54%	17.98%	2.53%	91.32%	5.42	0.03	175.84	79.49%
	Co-testing (cytology LSIL + & HPV any type positive)	90.39%	52.23%	75.63%	76.82%	47.77%	9.61%	75.93%	1.89	0.18	10.28	42.62%
	Co-testing (cytology LSIL + & HPV HR positive)	87.68%	58.21%	76.05%	75.74%	41.79%	12.32%	75.96%	2.1	0.21	9.91	45.89%
	Co-testing (cytology LSIL + & HPV 16/18 positive)	80.12%	84.42%	88.62%	73.73%	15.58%	19.88%	81.83%	5.14	0.24	21.85	64.55%
Histology HSIL+	Cytology ASCUS+	98.49%	43.77%	16.68%	99.61%	56.23%	1.51%	49.38%	1.75	0.03	50.83	42.26%
	Cytology LSIL+	93.36%	57.35%	20.01%	98.70%	42.65%	6.64%	61.04%	2.19	0.12	18.92	50.71%
	Cytology ASC-H+	75.72%	97.85%	80.06%	97.24%	2.15%	24.28%	95.58%	35.15	0.25	141.63	73.56%
	HPV anytype	92.22%	41.64%	15.73%	97.84%	58.36%	7.78%	46.98%	1.58	0.19	8.46	33.86%
	HPV HR positive	89.90%	49.31%	17.32%	97.64%	50.69%	10.10%	53.59%	1.77	0.2	8.66	39.21%
	HPV 16,18 positive	54.47%	84.94%	29.94%	94.05%	15.06%	45.53%	81.72%	3.62	0.54	6.75	39.41%
	Co-testing (cytology ASCUS + & HPV typing positive)	99.55%	21.88%	13.51%	99.75%	78.12%	0.45%	30.36%	1.27	0.02	61.54	21.43%
	Co-testing (cytology ASCUS + & HPV 16,18 positive)	99.14%	37.98%	15.51%	99.74%	62.02%	0.86%	44.28%	1.6	0.02	70.67	37.12%
	Co-testing (cytology ASCUS + & HPV HR positive)	99.48%	25.96%	13.37%	99.77%	74.04%	0.52%	33.53%	1.34	0.02	67.66	25.44%
	Co-testing (cytology LSIL + & HPV any type positive)	98.31%	28.70%	14.44%	99.29%	71.30%	1.69%	36.29%	1.38	0.06	23.46	27.02%
	Co-testing (cytology LSIL + & HPV HR positive)	97.94%	33.85%	14.54%	99.31%	66.15%	2.06%	40.46%	1.48	0.06	24.31	31.79%
	Co-testing (cytology LSIL + & HPV 16/18 positive)	95.88%	50.32%	18.15%	99.07%	49.68%	4.12%	55.01%	1.93	0.08	23.55	46.19%
	Co-testing (cytology ASC-H + & HPV any type positive)	97.00%	41.00%	17.02%	99.10%	59.00%	3.00%	47.21%	1.64	0.07	22.46	38.00%
	Co-testing (cytology ASC-H + & HPV HR positive)	95.70%	48.63%	17.65%	98.99%	51.37%	4.30%	53.48%	1.86	0.09	21.09	44.33%
Co-testing (cytology ASC-H + & HPV 16/18 positive)	87.80%	83.63%	38.16%	98.35%	16.37%	12.20%	84.06%	5.36	0.15	36.78	71.43%	

to December 2017 according to the Helsinki declaration and was approved by the “AT-IKON” University Hospital Bioethics Committee (decision id: 5/14-6-13) and the Bioethics Committee of the University Hospital of Thessaloniki (decision id: 6/12-8-14).

In Greece women are usually screened in an opportunistic basis. Normally, every one to two years a Pap smear

is obtained at a gynecologic clinic or a private practice. Women with a severe or sequential mild cytological abnormalities, would be referred for further assessment to colposcopy referral centers. All women who have been referred and attended University Hospital colposcopy clinics, from the aforementioned geographical areas of Greece (Attica, North West, and Northern Greece) for further assess-

Table 7. — HPV typing metrics for the categories indicative to the status of the cervix.

Histology	n	Any type 16 or 18 positivity		HR existence		LR existence		Nosubtypes		No HR	No LR	Multiple infections	
		n	%	n	%	n	%	n	%	Mean ± SD	Mean ± SD	Mean ± SD	%
		NEGATIVE or CN	2260	1033	45.71%	243	10.75%	899	39.78%	342	15.13%	0.8 ± 1.19	0.62 ± 0.96
LSIL or CLIN. LSIL	2853	1951	68.38%	527	18.47%	1693	59.34%	743	26.04%	1.3 ± 1.3	0.98 ± 1.07	0.32 ± 0.62	52.13%
HSIL	551	512	92.92%	297	53.90%	501	90.93%	105	19.06%	1.73 ± 1.2	1.5 ± 1.02	0.23 ± 0.52	50.00%
CxCa	51	45	84.91%	32	60.38%	42	79.25%	6	11.32%	1.08 ± 0.7	0.96 ± 0.68	0.11 ± 0.32	20.00%
Grand Total	5717	3541	61.94%	1099	19.22%	3135	54.84%	1196	20.92%	1.14 ± 1.28	0.88 ± 1.05	0.26 ± 0.56	48.52%

* The percentage of multiple infections was calculated on the total number of HPV infected cases for every histological grouping.

ment of any cytological abnormality, from September 2009 to August 2016 were enrolled in the study.

At presentation, a second new Liquid Based Cytologic (LBC) sample was obtained for cytological and biomolecular analysis for HPV DNA typing. In addition, women were asked to complete an anonymous questionnaire regarding their demographic characteristics, sexual life style aspects. Specifically: marital status, parity, smoking habits, education level, age of the first sexual intercourse, number of sexual partners, and frequency of condom use were investigated. The authors excluded individuals who refused to participate in the study or had inadequate sample for cytologic interpretation at initial evaluation.

Cytological and ancillary HPV-related molecular tests were conducted on LBC material, obtained before the colposcopic examination. All Pap smears were prepared according to routine process of cytological examination and the remaining material in the ThinPrep vial was used for HPV typing. Papanicolaou smears were assessed by experienced cytopathologists in the two involved University Cytopathology Departments.

Cytological reporting was concordant with the revised Bethesda classification system (TBS2001 system) [53, 54]. HPV DNA typing was performed with the CLART kit, which simultaneously detects 35 different HPV genotypes by PCR amplification of a fragment within the highly conserved L1 region of the virus [55].

In all cases where cervical biopsies were available, these were obtained under colposcopic guidance. The biopsy material was either a punch biopsy/ies, or cone biopsy in the form of Large Loop Excision of the Transformation Zone (LLETZ) cone specimen, in those cases or departments in which “see and treat” policy was considered most appropriate. Colposcopies and excisional procedures were always performed by experienced and accredited colposcopists (more than ten years in practice and certified by a relevant body), in each participating center. In all nulliparous women, when colposcopic impression was suggestive for high grade disease, punch biopsies were taken, and if the histology report was confirming the presence of CIN2+, women were planned for excisional treatment in a second visit at the end of their next menstrual period [56]. In women with colposcopic impression of moderate or se-

vere disease who had completed their childbearing, a “see and treat” approach was advocated.

Histological samples were fixed and prepared according to standard histopathology procedures. Histological reporting was based on the three-tiered CIN system; this assessment yielded the final correct diagnosis and was considered as the golden standard. In cases where both punch biopsies and LLETZ were available, the most severe diagnosis of the histology samples was considered as the final histological diagnosis.

Ideally, based on the study design, where cytological, biomolecular and colposcopic findings in addition to the demographic data were the baseline data for the final analysis, histological confirmation of any lesion was desirable for all study participants. However, in the final data set, histology was un-available in cases where obtaining biopsy material was considered unnecessary (e.g. newly diagnosed LSIL in young nulliparous women, cytological and colposcopic negative cases, individuals that were referred for further evaluation due to minor abnormal conventional cytology but with LBC as well as a negative colposcopy, or cases where both the cytology and colposcopy were suggestive of low-grade disease. For the study purposes, those cases were classified in sub-categories, as illustrated in Table 1.

Individuals with normal cytology, normal colposcopic findings and negative HPV DNA typing were considered as being histologically negative (clinically negative). Cases where HPV DNA typing tested positive for any HPV subtype (normal cytology and colposcopy) were considered as clinically LSIL. Cases with ASCUS or LSIL cytology and simultaneous colposcopic impression of low-grade disease (HPV or CIN1 or LSIL), and without histological confirmation, were considered as histologically LSIL (clinically LSIL).

Considering that histology or colposcopy outcomes were mandatory to define the “true” cervical status, (at a particular time) in the final analysis of the data, we included 6,527 cases fulfilling the inclusion criteria. The matrix correlating the cytological with the histological findings (including clinically negative and clinically LSIL cases) is presented in Table 2.

Results

Results of the demographic data obtained are presented in Table 3. The mean age for the < CIN2 (normal and LSIL) cases group ($n = 4,870$) was 35.53 ± 11.39 years in comparison to the CIN2+ (HSIL and cancer) group ($n = 569$), which was 36.65 ± 9.84 years (mean age). A statistically significant difference between these two groups was found, indicating a lower likeliness for harboring high grade cervical disease for younger women (difference = 1.06, S.E. = 0.498, 95% CI = 0.0839-2.036, $t = 2.129$, $p < 0.05$).

The mean age of the first sexual intercourse for the < CIN2 cases ($n = 2,980$) was 18.58 ± 2.68 years while for the CIN2+ group ($n = 490$) was 17.99 ± 2.27 years. A statistically significant difference between the two groups shows that coitarche at younger age accumulates a higher risk for high grade cervical lesions (difference = -0.59 years, S.E. = 0.131, 95% CI: 0.846 to -0.334, $t = -4.518$, $p < 0.0001$).

The mean number of sexual partners for women with lesions less severe than CIN2 ($n = 2867$) was 4.34 ± 5.04 , while for women with CIN2+ ($n = 477$) was found 6.17 ± 7.78 , respectively, ($p < 0.0001$). In other words, the fewer lifetime sexual partners, the lesser the risk for CIN2+.

At the analysis of consistent condom use (80% to 100% at lifetime sexual intercourses), no difference was observed between both groups.

The comparison of proportions illustrated a statistically significant difference between the groups of married women ($p < 0.05$) with less than CIN2 and married women with CIN2+ lesions.

Out of 333 < CIN2 women, 5.40% had had below the basic educational level, 31.24% had received basic education (high school), and 63.36% had received a University Level Degree or Technological Educational Institute training. Furthermore, out of the 103 women with CIN2+, 8.74% had received below the basic level education, 38.84% received the basic education level and the remaining 52.42% University or Technical Education Institute's degrees. These figures address a significantly higher possibility for CIN2+ in women with lower education level ($p < 0.05$). In women that had received basic education, there was no significant risk for CIN2+.

For the group of smokers and non-condom users, a statistically significant difference regarding the severity of the dysplasia was observed, corresponding to 27.34% for <CIN2 cases (210/768), in comparison to 42.37% for CIN2+ cases (100/236), (95% CI: 7.843% to 22.314%, Chi-squared = 18.411, $p < 0.0001$).

For a number of years, HPV typing has been implemented as an ancillary technique for cytology. However, accumulating data support the role of this test as a stand-alone that might replace traditional cytological screening in the future. We then looked at the performance metrics of the Papanicolaou test as a single examination test, vs. when it is combined with HPV typing. Aiming to detect abnormal cases, the Papanicolaou test was set as positive when the results was reported ASCUS+, and in a similar way the histol-

ogy was set as positive when the outcome was LSIL+ (i.e. CIN1+ in histology) or clinically LSIL. Inadequate cases either in histology or in cytology were excluded. The correlation matrix between histology and cytology is presented in Table 4.

As for co-testing assessment, a case was considered "negative" when both the cytological results and the HPV DNA typing were reported negative, and "positive" when either cytology was reported ASCUS+ or HPV typing was tested positive for any HPV subtype (from the 35 subtypes being detectable by the CLART2 assay applied in this study). Cases with inadequate samples (either cytology or HPV typing), were excluded. The relevant correlation matrix is presented in Table 5.

A variety of different triage tests and combinations of tests, for the detection of cases for both the histological cut offs (LSIL+/CIN1+ and HSIL+/CIN2+) were implemented in this study. Specifically, for histologically confirmed LSIL+ cases (i.e. histologically normal vs. abnormal) and for HSIL+ (i.e. for cases requiring immediate colposcopy); several approaches could be proposed, depending on the cut-off in cytology and the HPV typing result. For these two histological cutoffs: i) the authors calculated the performance indexes of cytology as single test for the following diagnostic thresholds: a) ASC-US+, b) LSIL+, and c) ASC-H+. ii) The performance indexes of HPV typing as single test for the following diagnostic combinations: a) for any HPV type, b) for HPV types 16/18, as well as for c. co-testing (see Table 6), (i.e. both cytology and HPV are positive).

For the detection of histologically normal vs. abnormal cases (histology = LSIL+), co-testing (cytology ASCUS+ and HPV DNA positive) illustrated a statistically significant higher sensitivity (2.07%) than Papanicolaou test alone (cutoff = ASC-US+) (95% CI: 1.57% to 2.581%, $p < 0.0001$). However, this came at the cost of reduced specificity, as for co-testing 1128 from 2274 women (49.60%) were considered positive, but were negative or clinically negative in histology. In contrast, when using only the Papanicolaou test, 229 out of 2661 women (8.61%) were wrongly classified as positive. The most appropriate methodology could be based on a balanced approach between sensitivity and specificity. The Youden index can be useful in this case. According to the results (Table 6) the higher Youden index (88.29%) was achieved for cytology ASCUS+ and the second higher index (79.49%) for the combination of cytology ASCUS+ and simultaneously HPV 16 or 18 positive. However, the gain in sensitivity was less than 1% and the loss in specificity more than 9%.

For the detection of histologically HSIL+ cases, the highest sensitivity (99.48%) was achieved for co-testing (cytology ASCUS+ and HPV HR positive); this was, however, at the cost of a very low specificity (25.96%). A balanced approach indicated that when applying cytology with cut-off ASC-H+, the Youden index is maximized (73.56%), (sensitivity = 75.72% and specificity = 97.85%), while co-

testing (cytology ASC-H+ and HPV 16 or 18 positive), yielded more balanced results: sensitivity = 87.80%, specificity = 83.63%, with a Youden index = 71.43%.

As stated, high risk HPV's are known to have a causative relation in cervical cancer development. Based on the biomolecular markers' data in this population, the authors assessed various parameters related to HPV typing and cervical biological status, and compared their positivity with the cytological, colposcopic, and histological phenotype. More specifically, the authors evaluated: i) the presence/existence of any HPV type, ii) the presence/existence of HPV types 16 and 18, iii) the existence of high-risk and iv) low-risk subtypes, v) the total number of subtypes in multiple infections, the number of vi) high risk subtypes and vii) low risk subtypes, as well as iix) the existence of multiple infections in relation to the groups indicating the existence of lesions in the cervix of various stages. The results are presented in Table 7.

Discussion

This is a large Greek study linking the largest national cervical pathology patient databases. Irrefutably, the absence of homogeneity of the data represents one of the study's main drawbacks. If the study was based only on women with a full dataset, with a more comprehensive approach, it might have rendered statistically significant correlations, but it would have reduced the amount of useful data. The approach to use data parts (especially for the demographic data) from which the studied quantities were available, enabled the extraction of useful conclusions.

From the analysis of the questionnaire regarding demographic characteristics, the authors conclude that women presenting with CIN2+ are approximately one year older compared to those individuals with CIN1 or no lesions. This difference, however, makes no difference in everyday practice. The authors believe that this finding could be of great importance in the management of women above 35 years, where a more stringent approach should be adopted by the national cervical screening program committee, with more frequent follow up visits and possibly more ancillary tests if abnormal smear is present at this age group. In an older study by Giannopoulos et al. based on data from 510 women with mild dyskaryotic smears, the authors used three age groups: 1–20 years, 20–25 years, and older than 25 years. They concluded that it was not possible to prioritize women for referral to colposcopy by age group alone, thus the pressure for colposcopies could not be reduced [57].

Another interesting finding from the analysis of demographic data was that women with normal cytology or CIN1 lesions had fewer lifetime sexual partners than women with CIN2+ lesions. In particular, the analysis demonstrated, 4.34 vs. 6.17 partners among groups ($p < 0.0001$). This finding is concordant with the results from the study by Chan *et al.* where the authors attempted to evaluate the factors predicting regression of untreated CIN2 and CIN3 in 93 women who were followed up for a six-month-period.

The findings indicated that women with five or fewer sexual partners had higher regression rates than women with more than five partners. This finding may be attributed to persistent HPV infection or infection with multiple HPV subtypes [58].

Despite no significant statistical difference was observed, less than 20% lifetime condom use in sexual intercourse was reported by about 35.6% of normal and CIN1 individuals compared to about 41.2% of CIN2+ cases. This rather contradicting finding might indicate that the study includes a population that is at a higher risk to harbor CIN2+ and uses less frequently condoms. As reported in a prospective controlled trial almost three decades ago, on a relatively small population with CIN1 lesions (46 cases), condom use cannot be considered as an effective treatment for CIN1, but it might have a protective role [59]. Consistent condom use has a protective role for HPV infection and a positive role on regression of cervical neoplasia [60]. [61] [63, 64]., although this risk reduction may not be observed after accounting the effects of confounding factors [62]. In this study no relation was found between the number of pregnancies and cervical status, when grouping normal, mild, and severe dyskaryotic cases (CIN1 or less and CIN2+). Similar results were published by Jensen et al. [65] concluding that HPV infections were not significantly associated with number of births and did not explain the strong relation of parity to the particular risk. However, controversial results were found in a recent study [66] of 312 women with persistent HPV infection where an increased risk for CIN3+ was estimated in women that had given birth compared to nulliparous population, and in the study of Ngaon and Yoshimura [61], who summarized that multiparity could increase the risk of cervical cancer [67].

Analyzing marital status data showed a difference in non-married women. Nonetheless, those percentages appear to have smaller difference in divorced women and were estimated subsequently 13% (CIN2+) and 9% (< CIN1). This finding is conformant with the study by de Graaff et al. suggesting that women with cervical cancer are more likely to be married and more particularly to be married at an earlier age [68]. Unfortunately, age at marriage was not included among the demographic characteristics data collected in our study.

When looking at the level of education as a potentially predicting factor for CIN2+, the analysis revealed that that women with higher education have lower probability for cervical neoplasias. The above data is concordant with a recent meta-analysis by Damiani et al., where the authors found that women with higher education were more conformant to test Papanicolaou screening, and alike the study by Franceschi et al., in which authors concluded that the excess of cervical cancer cases were found in women with a lower socio-economic status [63, 69, 70]. Financial constraints *might* represent another possible barrier to regular cervical screening.

Smoking was not associated with cervical neoplasia in

this study, as about 65% of women with normal or CIN1 lesions were smokers and a similar percentage (69%) was found within CIN2+ cases. Perhaps, this can be attributed to the findings of a recent study by Chatzistamatiou *et al.* indicating that smoking is not related to higher probability for E7 protein positivity on HR HPV positive women [71]. However, when smoking is combined with absent or minimal condom use, then it might represent a high risk factor for CIN2+ lesions.

Concerning the accuracy of the several implemented diagnostic tests, the Papanicolaou test, HPV typing, as well as both combined were analyzed under different cutoffs, as these could be considered as useful options depending on the clinical set-up and the available resources. When seeking for balanced sensitivity and specificity, aiming to identify women with histological CIN1+, then cytology as single test at the cut-off of ASCUS+, performs adequately (sensitivity = 96.90%, specificity = 91.39%). The addition of HPV typing could offer a small diagnostic advantage only if the simultaneous existence of types 16 and 18 were included, however, the gain in sensitivity was rather small (increment around 0.5% and sensitivity 97.47% (Table 6), but this comes at the cost of specificity (reduction from 91.39% to 82.02%, Table 6). Despite the fact that nowadays HPV 16 and 18 testing is rather inexpensive is offered in a lower cost and in a plethora of approved commercial kits, an extensive application could overload colposcopy rooms.

In terms of identifying women with histological HSIL+ (i.e. CIN2+), then, cytology, as a single diagnostic test, for the cut off value of ASC-H appeared to have a sensitivity of 75.72% and a specificity 97.85% as in the case of CIN1+ discrimination, the co-existence of HPV 16 and 18 increased sensitivity to 87.80% (more than 10 percentage units) at the cost specificity reduction to 83.63% (Table 6). Both woman age and parity status have an important role in subsequent management, since women of older age as well as women that have completed childbearing may proceed immediately to colposcopy and offered a LLETZ procedure in a “see and treat” setting. Otherwise a more conservative approach should be adopted, with strict cyto-colposcopic surveillance in short regular intervals.

A rather high number of carcinomas emerged in this study population (59/6527=0.09%, Table 2), and this finding could be possibly attributed to the following factors: A) a large proportion of participating women were referred to gynaecology/colposcopy centers due to abnormal cytology (i.e. presence of cervical pathology). In other words, the studied population could not be considered as a “general screened population” where CxCa incidence rates are estimated approximately 17.8 per 100,000 women. B) All colposcopy clinics were affiliated with large, referral University centers in which CxCa rates have always been higher, C) CxCa incidence and morbidity might actually be on the rise in Greece, with higher rates than what the world statistics suggest, attributed to high rates of immigration, a lack of

organized cervical cancer screening programme, absence of cancer registry, and the very low HPV vaccination population coverage [72-74].

From the participating women (n = 6527) that harbored carcinomas (n = 59) (see Table 2), 51 (out of 5,717) had valid HPV DNA readings (see Table 7); in particular 13 adenocarcinomas and 38 SCC's. Twelve out of 13 adenocarcinomas were positive in HPV typing (92.31%) and from the remaining 38 SCCs, 33 cases had positive HPV typing (86.84%). The difference in the percentages of HPV positivity between adenocarcinomas and SCCs cases was not statistically significant ($p > 0.05$). Despite the small number of carcinomas, this finding concurs with other studies; in a larger study by Tjalma *et al.* focusing on 3,162 women with invasive cervical carcinoma, 91.8% of cases were HPV positive [75]. If an HPV test is based solely on 16 and 18 subtypes then the percentage of missed carcinomas is 39.62% while a test based only on HR only types misses 20.75% of CxCa cases (Table 7), a fact conformant with other studies suggesting that genotyping solely for HPV 16 and 18 can miss the majority of patients with LSIL who are destined to progress to HGSIL [76]. Similarly, the percentage of missed HSIL cases if a triage method is based only on HPV typing alone rates 7.08%, 9.07% and 46.10% when the test is based on the detection of: any HPV type, any HR type and types 16 or 18 respectively (Table 7).

Conclusions

This study analyzed information that can be the basis for an organized and documented cervical pathology registry in Greece, which would represent an integral part of a national cancer registry.

The results of this study indicated that women's demographic characteristics show promise in identifying the risk and potential for cervical precancerous lesions, and that the time-honored test Papanicolaou still represents an important diagnostic test. When LBC is coupled with HPV typing enhanced performance characteristics are anticipated; these outcomes might be of great clinical importance and serve in several applications. However, extensive health economics studies are required in order to determine the cost-benefit ratio of these combinations in various health systems and populations settings.

Future studies based on this dataset could be the assessment of expression profile of cytology, HPV typing and cotesting performance for women in the vulnerable below 30-year age group, as well as the construction of models that input examination and demographic data in order to identify more accurately women at high risk for persistent hr-HPV infection.

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Conflict of interest

The authors declare no conflict of interest.

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