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Diagnostic accuracy of colposcopy in relation to human papillomavirus genotypes and multiple infection

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HIGHLIGHTS

- Colposcopy had similar accuracy in the detection of CIN3+ in HPV 16+ and in other infections.
- Multiple high-risk HPV infection did not affect colposcopic accuracy for CIN3+.
- Colposcopic diagnostic accuracy for women infected by low-risk HPV was poor.

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ABSTRACT

Objective. The aim of this study is to evaluate the diagnostic accuracy of colposcopy for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) in relation to the detection of human papillomavirus (HPV) type 16 and multiple HPV infection.

Methods. A cohort study of 2526 subjects attending a colposcopic service because of cytological abnormalities. HPV genotypes were identified using the INNO–LIPA genotyping system.

Results. The final colposcopic/pathological diagnoses were as follows: 1282 (50.8%) negative, 709 (28.1%) CIN1, 169 (6.7%) CIN2, 318 (12.6%) CIN3 and 48 (1.9%) invasive cervical cancer, respectively. Among women with ASCUS/LSIL, assuming any colposcopic abnormality as a cut-off, there were no significant differences in the sensitivities (83.8%, 95% CI = 76–89.6 as compared to 84.1%, 95% CI = 73.2–91.1, $p = 0.9$) and ROC curves (0.61, 95% CI = 0.58–0.65 as compared to 0.59, 95% CI = 0.54–0.64, $p = 0.5$) in the detection of CIN3+ lesions between subjects with single and multiple high-risk infection, and between subjects infected by HPV16 (83.1%, 95% CI = 73.7–89.7, ROC = 0.59, 95% CI = 0.54–063) or other high-risk HPVs (84.7%, 95% CI = 75.6–90.8, ROC = 0.62, 95% CI = 0.58–0.66, $p = 0.8$ and $p = 0.6$ compared to HPV16). After correction for confounders, the odds ratios of CIN3+ associated with any abnormal colposcopic findings were 2.47 (95%CI = 1.44–4.23, $p = 0.001$) among HPV16 positive, 3.34 (95% CI = 2.16–5.42, $p < 0.001$) among other high-risk HPVs and 1.3 (95% CI = 0.72–2.48, $p = 0.36$) among subjects with negative/low-risk HPVs.

Conclusion. In routine clinical practice, multiple infection or HPV16 positivity did not affect colposcopic accuracy in the diagnosis of CIN3+ lesions. The sensitivity of colposcopy was poor among subjects who were uninfected or infected by low-risk HPV genotypes.

Introduction

Referral to colposcopy for the screening of cervical intraepithelial neoplasia (CIN) is recommended for all women with cervical cytology diagnoses of high-grade squamous intraepithelial lesions (HSIL) or with atypical squamous cells where high grade squamous epithelial lesions cannot be excluded (ASC-H) [1,2]. Colposcopy is also recommended for HPV-positive women with atypical squamous cells of uncertain significance (ASCUS) or low grade SIL (1–2) on cervical cytology. Previous studies have hypothesised that the sensitivity of colposcopy in the detection of high grade CIN could be influenced by several factors such as the age of the subjects, extent of the lesion, HPV status and HPV genotype [3,4]. Infection with multiple HPV types is very common among women with CIN and seems to be associated with larger cervical lesions, more severe cytological and histological abnormalities and more persistent HPV infection [5–10]. To date, little is known regarding the relative effect of single and multiple high risk

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HPV infection on the diagnostic accuracy of colposcopy in routine clinical practice [11,12]. In addition, a previous study of cervicophotographs obtained from patients participating in the ALTS trial suggested that colposcopic accuracy could be influenced by HPV genotypes, being better for HPV16 than for other high risk HPVs [13]. Given the diffusion of HPV tests in primary screening and triage of ASCUS, the evaluation of the accuracy of colposcopy in the diagnosis of CIN in relation to multiple infection or to the presence of HPV 16 or other genotypes could provide important information to be used in cervical screening programs or in colposcopic referral policies. In addition, information on the viral factors affecting the accuracy of colposcopy could be used in clinical practice for tailoring decisions about biopsy procedures.

The purpose of the present study was to evaluate the accuracy of colposcopy according to the presence and number and type of HPVs detected in a cohort of women attending a colposcopic clinic because of cervical cytological abnormalities.

Patients and methods

The initial study population included all women aged between 21 and 65 years who attended the colposcopy clinic of the Department of Obstetrics and Gynecology of the IRCCS Fondazione Policlinico San Matteo, Italy, from 2009 to 2012 because of an abnormal Pap smear. Subjects were referred by the cytological screening service of our Department, from private practice, and from the screening services of external institutions. Exclusion criteria included pregnancy, HPV test or treatment for CIN in the last year, total hysterectomy, lack of a recent (1 month) Pap smear, and use of vaginal medication in the previous 2 days. The Institutional Review Board of our Hospital approved the study and informed consent was obtained from all subjects. All patients were treated according to an established protocol including HPV DNA detection and typing and colposcopy with targeted biopsies. Cervical samples for HPV typing were obtained immediately before colposcopy. After speculum examination, scrapes were taken with a cervix brush, suspended in ThinPrep-PreservCyt Solution (Cytic Corporation, Marlborough, MA, USA), and stored at 4 °C. DNA extraction was performed within 5 days form sampling using an automatic instrument (Magtration system 12 GC, VODEN) based on paramagnetic particles. HPV sequences from the L1 region were amplified by polymerase chain reaction (PCR) using SPF10 primers in a 50 μl final reaction volume for 40 cycles. Appropriate positive and negative controls were introduced for each set of reactions. Concurrent amplification of beta globin sequences was used as a control for assessment of DNA quality. HPV type-specific sequences were detected by the line probe assay INNO-LiPA HPV genotyping assay, version EXTRA (Innogenetics NV, Ghent, Belgium), according to the manufacturer's instructions. The EXTRA version of the assay allows the simultaneous and separate detection of 18 high-risk HPV types (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, with proven carcinogenicity and Group I of the classification of International Agency for Research on Cancer (IARC); HPVs 26, 53, 66, 68, 73, and 82, with probable carcinogenicity and Group II IARC classification), 7 low-risk HPV types (6, 11, 40, 43, 44, 54 and 70), and 2 unclassified-risk HPV types (69/71 and 74) [14]. Hybridisation patterns were automatically analysed by the Liras system and checked by two independent readers. Overall, 27 different viral types were identified. A standardised colposcopic examination was performed immediately after cervical brushing for HPV typing by three expert gynaecology residents certified by the Italian Society of Colposcopy. Multiple (2 to 4) targeted cervical biopsies were obtained in all cases where CIN was suspected on colposcopy and in all cases of high-grade squamous cervical lesions (HSIL) irrespective of colposcopic impression. Endocervical curettage was performed when the extent of the lesion or the squamocolumnar junction was not entirely visible or in the case of atypical glandular cells (AGC) on Pap smear. Colposcopists were asked to describe the visibility and the characteristics of the transformation zone (types 1, 2, 3), the presence of grade 1 (minor) (smooth surface, irregular outer border, slight aceto-white change, slow to appear, fine punctation and regular mosaic, mild or speckled iodine partial positivity) or grade 2 (major) (sharp border, dense aceto-white change which appears early, coarse punctation and wide irregular mosaics, iodine negativity) abnormal colposcopic findings, and the size of the lesion (0, <25, 25–50, 51–75 or >75% of the cervix) [15,16]. Cytological results were reported according to Bethesda system terminology [17] whereas CIN refers to histological diagnoses. In the analysis of data, we used either histological diagnosis of the punch biopsy or, when more severe, the diagnosis after cone biopsy obtained by loop electro-excision procedure (LEEP) or cold-knife excision. In statistical analysis, outcome was defined as negative when colposcopy and/or cervical biopsies were negative, or as CIN1, CIN2, CIN3, and invasive squamous or adenocarcinoma of the cervix. HPV infection was classified as negative, low risk (single or multiple types), single high risk types or multiple high risk types (with or without concurrent low risk HPV types). After diagnostic workup and treatment, patients referred from the cytological screening service of our Department were enrolled in a follow-up programme including: a) observation, colposcopy, and/or cytology every 6–12 months for subjects with negative colposcopic impression and/or negative histological findings after an abnormal Pap smear; b) observation, colposcopy, and cytology every 6 months for CIN1 lesions; and c) HPV test coupled with colposcopy and cytology every 6 months for treated CIN2–3 lesions.

Normality assumption for continuous variables was assessed by Shapiro–Wilk test. Data were analysed by Kruskal–Wallis analysis of variance and Bonferroni corrected post-hoc test to compare continuous and chi-square test to compare categorical variables, respectively. The Bonferroni method for multiple comparisons was used to adjust chi-square test results in multiway contingency tables. Concordance between severity of colposcopic/pathologic classification and grading of colposcopic impression was computed by Spearman rank correlation coefficient. Chi-square for trend was used to test for trend on ordinal variables. Generalised odds ratio and 95% confidence interval were used to test the difference between two ordinal variables in tests for trend. The performance (sensitivity, specificity and receiver-operating characteristic (ROC) curve) of colposcopic examination in the detection of CIN 3 lesions or worse (CIN3+) was evaluated for different strata of Pap smear and HPV test results.

Since the performance of colposcopy could be influenced by several confounders such as Pap smear results at entry or age of the patients, the association between colposcopic impression and colposcopic/pathologic classification was also tested by logistic regression analysis. Logistic equations included CIN3+ as outcome variable, and colposcopic impression (negative, any abnormality), Pap smear results (ASCUS/LSIL, HSIL/ASC-H, ACG), hormonal contraception (yes, no), smoking (yes, no) and age as explanatory variables. Logistic regression was repeated for the strata of HPV infection (negative/low risk, high risk HPV infection, HPV16 positive, other high risk HPVs). Data were analysed with Stata/MP 10 for Windows (StataCorp LP, College Station, TX, USA).

Results

During the period of the study, 2658 women with abnormal Pap smear were seen at our colposcopy clinic and 2581 (97.1%) agreed to participate in the study. Sixty-five additional women were excluded because of inadequate HPV sampling (53 subjects) or inadequate colposcopic examination (12 subjects) leaving 2526 subjects for the final analysis.

The final colposcopic/pathological diagnoses were as follows: 1282 subjects (50.8%) were negative, 709 (28.1%) subjects were diagnosed with CIN 1, and 169 (6.7%), 318 (12.6%) and 48 (1.9%) subjects were diagnosed with CIN 2, CIN 3 and invasive cervical cancer, respectively. Negative diagnoses included 708 (55.2%) subjects with negative cervical biopsies and 574 (44.8%) subjects in whom colposcopically-directed biopsies were considered unnecessary. Invasive cervical cancers included 18 cases of adenocarcinoma and 30 of squamous