IJC
International Journal of Cancer

# Human papilloma virus genotyping for the cross-sectional and longitudinal probability of developing cervical intraepithelial neoplasia grade 2 or more

Annarosa Del Mistro[1], Rachael Adcock[2], Francesca Carozzi[3], Anna Gillio-Tos[4], Laura De Marco[4], Salvatore Girlando[5], Raffaella Rizzolo[6], Helena Frayle[1], Morena Trevisan[4], Cristina Sani[3], Elena Burroni[3], Paolo Giorgi Rossi[7], Jack Cuzick[2], Guglielmo Ronco[6] and the New Technologies for Cervical CancerWorking Group

[1] Veneto Institute of Oncology IOV—IRCCS, Padua, Italy
[2] Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Queen Mary University of London, London, UK
[3] Institute for Cancer Study and Prevention (ISPO), Florence, Italy
[4] Cancer Epidemiology—CERMS, University of Turin, Italy
[5] Pathology Unit, S. Chiara Hospital, Trento, Italy
[6] Center for Cancer Epidemiology and Prevention (CPO), Turin, Italy
[7] Epidemiology Unit, AUSL Reggio Emilia, IRCCS, Reggio Emilia, Italy

**Human papilloma virus (HPV) testing is more sensitive but less specific than cytology. We evaluated stand-alone genotyping as a possible triage method. During a multicentre randomised controlled trial comparing HPV testing to conventional cytology, HPV-positive women were referred to colposcopy and followed up if no high-grade lesion was detected. HPV-positive samples were genotyped by GP5+/GP6+ primed polymerase chain reaction followed by reverse line blot. Genotypes were hierarchically ordered by positive predictive value (PPV) for CIN grade 2 or more (CIN2+), and grouped by cluster analysis into three groups (A, B and C in decreasing order). Receiver operating characteristic curves were computed. Among 2,255 HPV-positive women with**

**Key words:** HPV, genotyping, triage, cervical screening

**Abbreviations:** CI: confidence interval (95%); CIN: cervical intraepithelial neoplasia; HPV: human papilloma virus; LBC: liquid-based cytology; NPV: negative predictive value; NTCC: New Technologies for Cervical Cancer; PCR: polymerase chain reaction; PPV: positive predictive value; RCT: randomized controlled trial; ROC: receiver operating characteristic

Additional Supporting Information may be found in the online version of this article.

**Conflict of interest**: J.C. is part of speakers buro/advisory board for BD, Abbott, Hologic, Trovagene and Cepheid. PGR as PI of an independent study funded by the Italian Ministry of Health, data owner, conducted negotiations with Roche diagnostics, Hologic, Qiagen, and Abbott to obtain reagents at reduced price or for free. The authors declare that they have no potential conflicts of interest.

The following are components of the New Technologies for Cervical Cancer Working Group: ***Turin***—N. Segnan, P. Giubilato (CPO Piemonte), B. Ghiringhello (Unit of Pathology, OIRM S. Anna), A. Sapino and M.G. Accinelli (Unit of Pathology, University of Turin), G. Maina and L. Pasero (Centre for cancer early Diagnosis and treatment, OIRM S. Anna); ***Trento***—P. Dalla Palma, E. Polla (Unit of Pathology, Ospedale di Trento); ***Veneto***—R. Trevisan (Istituto Oncologico Veneto–IRCCS, Padova), M. Zorzi, C. Fedato and S. Baracco (Registro Tumori and Regione Veneto), E. Insacco, D. Minucci, M. Matteucci (Unity of Gynaecology, Azienda Ospedaliera di Padova), G.L. Onnis (Department of Pathology, University of Padua), R. Colombari (Unit of Pathology, Ospedale di S. Bonifacio, AUSL Verona); ***Emilia-Romagna***—M. Manfredi (Centro screening, AUSL Bologna), G.P. Casadei, P. Pierotti (Unit of Pathology, Ospedale Maggiore, AUSL Bologna), G. Collina (Unit of Pathology Ospedale Bellaria, AUSL Bologna), P. Schincaglia, M. Serafini and B. Vitali (Centro Prevenzione Oncologica, AUSL Ravenna), M. Aldi (Unit of Pathology, Presidio Ospedaliero di Faenza, AUSL Ravenna), S. Folicaldi, R. Nannini, G. Galanti and M. De Lillo (Unit of Pathology, Presidio Ospedaliero, AUSL di Imola), C. Naldoni (Centro di riferimento screening - Assessorato alla Sanità–Regione Emilia-Romagna, Bologna)**;** ***Florence***—M. Confortini, A. Iossa, S. Ciatto, M.P. Cariaggi, S. Cecchini, M Zappa (ISPO Florence) and G.L. Taddei (Unit of Pathology, University of Florence); ***Lazio***—S. Brezzi, P. Raggi and E. Gomes (Local Health Unit, Viterbo, Italy), A. Pellegrini and M.L. Schiboni (Ospedale S. Giovanni, Rome), P. Borgia and F. Chini (ASP Lazio).

**Grant sponsor:** Ministero della Salute; **Grant sponsor:** FP7 Health; **Grant number:** HEALTH-F3-2013–603019; **Grant sponsor:** Associazione Italiana per la Ricerca sul Cancro; **Grant number:** IG14119

**DOI:** 10.1002/ijc.31326



**History:** Received 11 Sep 2017; Accepted 6 Feb 2018; Online 9 Mar 2018

**Correspondence to:** Guglielmo Ronco, Center for Cancer Epidemiology and Prevention (CPO), Via Cavour 31, 10123 Torino, Italy, Tel.: 39011-633-3850, E-mail address: guglielmo.ronco@cpo.it

genotyping, 239 CIN2+ (including 113 CIN3+) were detected at baseline or during a 3-year follow-up. HPV33 had the highest PPV with CIN2+ and CIN3+ as the endpoint and when considering lesions detected at baseline or also during follow-up. HPV16 and HPV35 were the second and third, respectively. Cross-sectional sensitivity for CIN2+ at baseline was 67.3% (95% CI 59.7–74.2), 91.8% (95% CI 86.6–95.5) and 94.7% (95% CI 90.2–97.6), respectively, when considering as "positive" any of the HPV types in group A (33, 16 and 35), A or B (31, 52, 18, 59 and 58) and A or B or C (39, 51, 56, 45 and 68). The corresponding cross-sectional PPVs for CIN2+ were 15.8% 95% (CI 13.2–18.7), 12.0% (95% CI 10.3–13.9) and 9.6% (95% CI 8.2–11.1), respectively. HPV33, 16 and 35 confer a high probability of CIN2+ but this rapidly decreases when adding other genotypes.

**What's new?**

The Human papilloma virus (HPV) infection genotype is an early predictor of cervical lesions. The current focus lies on high-risk genotypes 16 and 18, frequently found in cancers. Here the authors studied the predictive value of all carcinogenic types and identified infections with HPV16, but also HPV33 and 35, as conferring very high risk for the development of high-grade cervical intraepithelial neoplasia (CIN2+). However, HPV genotyping alone did not reliably triage women, and combinations with other methods are recommended.



## Introduction

Cervical cancer screening based on HPV testing allows earlier diagnosis of persistent high-grade cervical intraepithelial neoplasia (CIN) than cytology-based screening[1] and is more effective in preventing invasive cervical cancer.[2] However, as the specificity of HPV testing for high-grade CIN is low,[1] a triage for selecting which HPV-positive women are referred to colposcopy is needed. All randomized controlled trials (RCTs) except one (NTCC, New Technologies for Cervical Cancer) referred to colposcopy only HPV-positive women with abnormal cytology or persistent HPV infection. This approach, compared to stand alone cytology, increased efficacy without increasing the biopsy rate.[2] Nonetheless, short-term test repeats are needed, which is disturbing for women[3] and entail appreciable loss to follow-up[4] so investigating other triage methods is of interest.

It has been shown that different oncogenic HPV genotypes[5] have different cross-sectional[6] and longitudinal[7] strengths of association with high-grade CIN and invasive cervical cancer. In particular, HPV16 has been shown to be strongly associated with both high-grade CIN and invasive cervical cancer.[8–10] HPV31, 33, 35, 52 and 58 are particularly associated to high-grade lesions.[8] HPV18 and 45 are not clearly associated with CIN3[8] but are the second and third most common types in invasive cervical carcinoma and are specifically associated with adenocarcinoma and glandular intraepithelial lesion.[11]

We used the biobank stored during the NTCC trial to study the cross-sectional and longitudinal association of HPV genotypes with high-grade CIN. Previously, partial genotyping for HPV16 and 18 has been studied as a triage method in association with cytology.[6,10] This strategy has already been included in the American multi-societal and United States Preventive Services Task Force (USPSTF) guidelines.[12,13] Here, we report the accuracy of stand-alone genotyping as a triage test.

## Materials and Methods

NTCC is an RCT, with two pre-planned recruitment phases, which was conducted in nine population-based cervical screening programs in Italy. Women aged 25–60 years who were not pregnant, had never underwent hysterectomy, had not been treated for CIN in the last 5 years and who were attending for a new routine cervical screening episode were randomly assigned, between February 2002 and December 2004, to conventional cytology (classified according to the Bethesda 1991 system and managed according to the standard protocol of each centre) or to HPV-based screening, either in combination with liquid-based cytology (phase 1)[14,15] or alone (phase 2).[16] Details on randomization and masking have been reported previously.[14–17]

Cervical specimens were collected using a plastic Ayre's spatula and a cytobrush, and eluted into the PreservCyt buffer (Hologic/Gen-Probe, San Diego, CA (phase 1) or in the standard transport medium (STM, Qiagen, Hilden, Germany) (phase 2). The presence of HPV in cervical specimens was evaluated by hybrid capture 2 (HC2, Qiagen) using probes designed to detect 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68).

NTCC is registered as an International Standard RCT, number ISRCTN81678807. We obtained central and local research ethics approvals.

### Colposcopy and post-colposcopy follow-up

During phase 1, all women in the HPV arm were referred to colposcopy if cytology was atypical squamous cells of undetermined significance (ASCUS) or more severe. In addition, HPV-positive women with cytology less than ASCUS were directly referred to colposcopy if aged 35–60 years,[14] while those aged 25–34 years were referred to colposcopy only if after 1 year HC2 remained positive or cytology was ASCUS or greater.[15] During phase 2, women in the HPV arm were referred for colposcopy if the HPV test was positive.[16]

Therefore, in the experimental arm, management at recruitment varied by phase and age.

As a rule, women with CIN2+ were treated and those <CIN2 followed up colposcopically. In the HPV arm, HPV-positive women were actively recalled after colposcopy for annual repeats of HC2 and liquid-based cytology (LBC), as long as HPV remained positive. They were referred to colposcopy if LBC was ASCUS or more severe.[17]

### Genotyping

Storage of residual cell samples after a positive HC2 test was set up during both study phases in Florence, Padua, Trento and Turin, while in Bologna, Imola, Ravenna and Viterbo, it was only performed during the second phase and in Verona, it was never performed. Residual cells from samples collected in PreservCyt were stored at −80°C following double PBS washing to remove methanol; aliquots (400 µl) from those collected in STM were taken before HC2 testing and directly stored at −80°C in their own solution.

The first HC2-positive sample of each woman was genotyped. Genotyping was performed, blindly to histology results, by GP5+/GP6+ primed polymerase chain reaction (PCR), followed by reverse line blot.[18,19] Although GP5+/GP6+ PCR could in principle detect >40 HPV types, in this analysis, HPV genotyping was restricted to the 13 high-risk HPV types targeted by HC2. In addition, although other methods were applied in samples where no HC2-targeted HPV types were identified,[20,21] their results are not considered in the present analysis. Typing results were not used for women's management.

### Endpoint assessment

Endpoints were histologically confirmed CIN2, CIN3 or invasive cervical cancer.

We recorded test results and histological findings from the computerized registration systems of participating screening centres. At the end of the recruitment phase, for women who had a histology originally diagnosed as CIN1 or more severe by the local pathology laboratory, all histological specimens taken within 1 year of referral to colposcopy were reviewed. For each woman, all histological slides, both from colposcopy-guided biopsies and treatments, were provided together, and the most severe diagnosis was used. Each case was randomly assigned to one or two pathologists randomly selected from a group of nine who were unaware of the original diagnosis, randomization or genotyping. Only morphological criteria were used. If a pathologist did not agree with the original diagnosis regarding the presence of CIN2+, then the information was discussed by at least two further pathologists. This consensus diagnosis was then used in all the published analyses of NTCC data. The same exercise was repeated at the time of the second screening round. The results of such reviews were previously reported.[14,16,17,22,23] Adenocarcinoma *in situ* was considered with CIN3. In addition, to obtain histological diagnoses performed outside the trial, after the end of the second round, we linked the database of recruited women to those of the cancer registries (covering all centres except Viterbo) and of the Pathology Units present in the catchment areas of NTCC.[17] Considering the women included in this analysis, 44 of 224 (19.6%) with histology locally diagnosed as CIN2+ at baseline or follow-up were downgraded to CIN1 or no CIN while 19 of 406 (4.7%) with histology locally diagnosed as CIN1 were upgraded to CIN2 or more severe. For 41 women with CIN2+ diagnosis, slides for review could not be retrieved and the local diagnosis was kept. Five other were added from cancer registries.

For this analysis, lesions detected at baseline were defined as those detected within 1 year from referral to colposcopy. Lesions detected thereafter were defined as detected during follow-up.

### Patients

This analysis was restricted to women in the experimental arm who received HPV-based screening ($n = 47{,}369$). To have colposcopic verification among almost all HPV-positive women, those aged 25–34 years recruited during phase 1 were excluded from all analyses ($n = 6002$), and a further 7,877 women from centres where samples for genotyping were not stored were excluded leaving 33,490 women. Among them, 33,249 women had a valid HC2 result and 2,498 were found to be HPV positive. A further 100 HC2-positive women were excluded from our analysis because their corresponding samples for genotyping were not available, and 143 women were excluded because they did not have colposcopy. The final sample for analysis was 2,255 HPV-positive women (Supporting Information Fig. S1). Of these, 1,685 had at least one HPV genotype targeted by HC2, while in the remaining either low-risk HPV types ($n = 302$) or no HPV types were detected by PCR ($n = 268$).

Of the 2,084 women who had no high-grade CIN detected at enrolment, 1,900 (91%) received further tests as part of the post colposcopy follow-up. Median duration of their follow-up was 1145 days (IQR 796–1473). Of the 1,900 women, 1,636 (86%) were actively followed up until either detection of high-grade CIN, negative for a HPV test or for at least 3 years, while 264 (14%) had a shorter follow-up without disease or resolution of HPV infection (not presented for retesting).

### Statistical methods

For each individual high-risk HPV genotype, prevalence and positive predictive values (PPV) for CIN2+ and CIN3+ were calculated. Hierarchical ranking of HPV types was obtained by iteratively determining the subsequent genotype with the highest PPV excluding cases with multiple infections with higher ranking types. Receiver operating characteristic (ROC) curves were plotted showing cumulative sensitivity and specificity accounting for the genotype hierarchy. A k-means cluster analysis was used to group the hierarchical ordered individual genotypes into three (highest risk A to lowest risk C) groups based on the hierarchical PPV values. Clusters were determined by iteratively calculating the sum

Table 1. Number of women with high-risk genotype, alone or with other types, and prevalence among study women

| Genotype | Number of women with genotype, alone or with other types | Prevalence in HPV+ women (%) |
|---|---|---|
| HPV 16 | 632 | 28.03 |
| HPV 31 | 291 | 12.90 |
| HPV 18 | 203 | 9.00 |
| HPV 56 | 181 | 8.03 |
| HPV 45 | 129 | 5.72 |
| HPV 58 | 119 | 5.28 |
| HPV 51 | 117 | 5.19 |
| HPV 39 | 94 | 4.17 |
| HPV 52 | 90 | 3.99 |
| HPV 59 | 72 | 3.19 |
| HPV 33 | 67 | 2.97 |
| HPV 35 | 50 | 2.22 |
| HPV 68 | 45 | 2.00 |

In this table, women appear many times in the case of multiple infections.

of squares for each cluster with the aim of minimizing the within cluster variation. Sensitivity, specificity and absolute risk of disease for women positive (PPV) and negative (1 minus negative predictive value [NPV]) for certain HPV types were calculated. Relative risks for CIN2+ and CIN3+, if positive for different genotypes, were also calculated. Diagnostic accuracy was computed for individual genotypes in cluster A (very highly predictive), then cumulatively for cluster B (highly predictive) and C (intermediately predictive). Associated 95% confidence intervals (95% CI) were computed based on the exact binomial distribution. Data were also computed for the HPV16 and 18 partial genotyping, since this is proposed by some commercially available tests.

Pretest–posttest probability (PPP) plots starting from the HPV test were constructed. For groups of genotypes, the post-positive test and post-negative test probabilities were the corresponding PPV and 1-NPV, respectively. The post-negative-HPV probability was computed including all the lesions detected up to the following screening round within NTCC (up to 3 years).

All analysis was conducted in Stata 13.1 (StataCorp, College Station, Texas).

## Results

Overall, 2,255 HPV-positive women with genotyping for the 13 types targeted by HC2 were included in the analysis. At enrolment, 171 (7.6%) women had CIN2+ histology (including 80 CIN3+), and among them, 9 CIN2+ (3 CIN3+) cases had no high-risk HPV genotypes detected. Furthermore, 68 CIN2+ were detected during the 3-year follow-up (including 33 CIN3+). Thus, 239 CIN2+ (113 CIN3+) cases were detected overall, of which, 13 CIN2+ (4 CIN3+) cases (5.44%) had no high-risk HPV types detected.

HPV16 was the most common genotype identified in this HC2-positive population, followed by HPV31 and 18, with 28.0%, 12.9% and 9.0% prevalences, respectively (Table 1). The number of women positive for each HPV genotype and of those with CIN2+ and CIN3+ detected at baseline and during follow-up, after adjusting for the type hierarchy, are reported in Table 2. For each endpoint and time period, genotypes are ordered by PPV and clustered. HPV33 was the genotype with the highest PPV, both when considering the cross-sectional and longitudinal analysis, and for both CIN2+ (22.4% and 25.4%, respectively) and CIN3+ (11.9% and 11.9%, respectively). HPV16 was always second and HPV35 third in the hierarchy, except for outcome CIN2+ in the longitudinal analysis, where the order was reversed. When excluding cases with disease detected at baseline, HPV35 was very predictive of CIN2+ during follow-up. When grouping genotypes by cluster analysis (Table 2), with CIN2+ at baseline as the endpoint, HPV types 33, 16 and 35 formed cluster A, the most predictive of high-grade lesions. The cumulative prevalence of cluster A was 32.2% of all HC2 positive women. HPV18, 31, 52, 58 and 59 were included in cluster B, while HPV 39, 45, 51 and 56 were included in cluster C. Types 18, 31, 58 and 59 were included in cluster B for all endpoints and type 52 for all except CIN3+ (baseline or follow-up). Conversely, HPV51 and 45 were always and HPV68 three out of four times (excluding CIN3+ at baseline) included in cluster C. Considering the original histological diagnoses provided locally had limited effect on the order of genotypes by PPV. In particular, the first 3 genotypes remained unchanged independently of the endpoint grade and of cross-sectional or longitudinal analysis. PPV slightly decreased as expected with a more misclassified endpoint.

When considering as "positive" any of the types in group A, cross-sectional sensitivity for CIN2+ at baseline was 67.3% (95% CI 59.7–74.2) (Table 3). This increased to 91.8% (95% CI 86.6–95.5) and 94.7% (95% CI 90.2–97.6) when adding cluster B (31, 52, 18, 59 and 58) and C (39, 51, 56, 45 and 68). The cumulative sensitivity was very similar for both endpoints, and for lesions detected at baseline and follow-up. The risk of being CIN2+ was 3.7% (95% CI 2.8–4.7)) for women negative to the genotypes in cluster A (1-NPV), and 1.5% (95% CI 0.8–2.5) for those negative to the genotypes in clusters A and B. The risk was similar for women who had group C types and for those who were HC2 positive but had no oncogenic type detected.

Among women positive for types included in clusters A, A or B and A or B or C, the probability of CIN2+ (PPV) was 15.8% (95% CI 13.2–18.7), 12.0% (95% CI 10.3–13.9) and 9.6% (95% CI 8.2–11.1), respectively, and for CIN3+, it was 8.7% (95% CI 6.7–11.0), 5.7% (95% CI 4.6–7.1) and 4.6% (95% CI 3.6–5.7), respectively.

When including lesions detected during follow-up (Table 4), the risk of CIN2+ if negative for HPV types in cluster A was

**Table 2.** Number of women and of those among them having or developing CIN2+ or CIN3+ who are positive for each new genotype in a hierarchical analysis.

| Cluster | Genotype | Women1 | CIN2+ | PPV (%) | Cumulative prevalence in HPV+ (%) | Cumulative PPV (%) | Cluster | Genotype | Women1 | CIN3+ | PPV (%) | Cumulative prevalence in HPV+ (%) | Cumulative PPV (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A. Endpoint CIN2+ at baseline | | | | | | | B. Endpoint CIN3+ at baseline | | | | | | |
| A | HPV 33 | 67 | 15 | 22.39 | 2.97 | 22.39 | A | HPV 33 | 67 | 8 | 11.94 | 2.97 | 11.94 |
| | HPV 16 | 616 | 93 | 15.10 | 30.29 | 15.81 | | HPV 16 | 616 | 52 | 8.44 | 30.29 | 8.78 |
| | HPV 35 | 44 | 7 | 15.91 | 32.24 | 15.82 | | HPV 35 | 44 | 3 | 6.82 | 32.22 | 8.87 |
| B | HPV 31 | 238 | 20 | 8.40 | 42.79 | 13.99 | B | HPV 58 | 100 | 4 | 4.00 | 36.67 | 8.10 |
| | HPV 52 | 71 | 5 | 7.04 | 45.94 | 13.51 | | HPV 59 | 58 | 2 | 3.45 | 39.25 | 7.80 |
| | HPV 18 | 139 | 9 | 6.47 | 52.11 | 12.68 | | HPV 68 | 38 | 1 | 2.63 | 40.93 | 7.58 |
| | HPV 59 | 45 | 3 | 6.67 | 54.10 | 12.46 | | HPV 31 | 227 | 4 | 1.76 | 51.00 | 6.43 |
| | HPV 58 | 88 | 5 | 5.68 | 58.00 | 12.00 | | HPV 18 | 132 | 2 | 1.52 | 56.85 | 5.93 |
| C | HPV 39 | 58 | 2 | 3.45 | 60.58 | 11.64 | | HPV 52 | 59 | 1 | 1.69 | 59.47 | 5.74 |
| | HPV 51 | 80 | 2 | 2.50 | 64.12 | 11.13 | C | HPV 39 | 57 | 0 | 0.00 | 62.00 | 5.51 |
| | HPV 56 | 125 | 1 | 0.80 | 69.67 | 10.31 | | HPV 45 | 93 | 0 | 0.00 | 66.12 | 5.16 |
| | HPV 45 | 87 | 0 | 0.00 | 73.53 | 9.77 | | HPV 51 | 75 | 0 | 0.00 | 69.45 | 4.92 |
| | HPV 68 | 27 | 0 | 0.00 | 74.72 | 9.61 | | HPV 56 | 119 | 0 | 0.00 | 74.72 | 4.57 |
| C. Endpoint CIN2+ at baseline or follow-up | | | | | | | D. Endpoint CIN3+ at baseline or follow-up | | | | | | |
| A | HPV 33 | 67 | 17 | 25.37 | 2.97 | 25.37 | A | HPV 33 | 67 | 8 | 11.94 | 2.97 | 11.94 |
| | HPV 35 | 50 | 12 | 24.00 | 5.19 | 24.79 | | HPV 16 | 616 | 67 | 10.88 | 30.29 | 10.98 |
| | HPV 16 | 610 | 121 | 19.84 | 32.24 | 20.63 | | HPV 35 | 44 | 5 | 11.36 | 32.24 | 11.00 |
| B | HPV 31 | 238 | 28 | 11.76 | 42.79 | 18.45 | B | HPV 39 | 74 | 5 | 6.76 | 35.52 | 10.61 |
| | HPV 52 | 71 | 7 | 9.86 | 45.94 | 17.86 | | HPV 58 | 99 | 5 | 5.05 | 39.91 | 10.00 |
| | HPV 39 | 65 | 6 | 9.23 | 48.82 | 17.35 | | HPV 59 | 55 | 2 | 3.64 | 42.35 | 9.63 |
| | HPV 58 | 96 | 8 | 8.33 | 53.08 | 16.62 | | HPV 18 | 137 | 5 | 3.65 | 48.43 | 8.88 |
| | HPV 18 | 125 | 10 | 8.00 | 58.63 | 15.81 | | HPV 56 | 135 | 4 | 2.96 | 54.41 | 8.23 |
| | HPV 59 | 44 | 3 | 6.82 | 60.58 | 15.52 | | HPV 31 | 208 | 6 | 2.88 | 63.64 | 7.46 |
| | HPV 56 | 127 | 8 | 6.30 | 66.21 | 14.74 | C | HPV 52 | 58 | 1 | 1.72 | 66.21 | 7.23 |
| C | HPV 51 | 78 | 3 | 3.85 | 69.67 | 14.19 | | HPV 45 | 90 | 1 | 1.11 | 70.20 | 6.89 |
| | HPV 45 | 87 | 3 | 3.45 | 73.53 | 13.63 | | HPV 51 | 75 | 0 | 0.00 | 73.53 | 6.57 |
| | HPV 68 | 27 | 0 | 0.00 | 74.72 | 13.41 | | HPV 68 | 27 | 0 | 0.00 | 74.72 | 6.47 |

PPV for each new genotype and cumulatively. Cumulative prevalence in HPV+ women. Genotypes are ordered by PPV for each endpoint.
[1]The number of women, CIN2+ and CIN3+ is computed considering only those not included in upper lines because they had multiple infections with previously considered genotypes. Each woman appears only once for each endpoint.

**Table 3.** Accuracy of individual genotypes and clusters for HC2 positive women[1]

| Endpoint | HPV types | $n/N$[2] | Sensitivity | Specificity | PPV | 1-NPV | Relative risk[3] |
|---|---|---|---|---|---|---|---|
| CIN2+ ($N = 171$) | HPV 33 | 15/67 | 8.8 (5.0, 14.1) | 97.5 (96.7, 98.1) | 22.4 (13.1, 34.2) | 7.1 (6.1, 8.3) | 3.1 (1.9, 5.0) |
| | HPV 16 | 95/632 | 55.6 (47.8, 63.1) | 74.2 (72.3, 76.1) | 15.0 (12.3, 18.1) | 4.7 (3.7, 5.8) | 3.2 (2.4, 4.3) |
| | HPV 35 | 7/50 | 4.1 (1.7, 8.3) | 97.9 (97.2, 98.5) | 14.0 (5.8, 26.7) | 7.4 (6.4, 8.6) | 1.9 (0.9, 3.8) |
| | Cluster A | 115/727 | 67.3 (59.7, 74.2) | 70.6 (68.6, 72.6) | 15.8 (13.2, 18.7) | 3.7 (2.8, 4.7) | 4.3 (3.2, 5.8) |
| | Cluster A or B | 157/1308 | 91.8 (86.6, 95.5) | 44.8 (42.6, 46.9) | 12.0 (10.3, 13.9) | 1.5 (0.8, 2.5) | 8.1 (4.7, 13.9) |
| | Cluster A or B or C | 162/1685 | 94.7 (90.2, 97.6) | 26.9 (25.0, 28.9) | 9.6 (8.2, 11.1) | 1.6 (0.7, 3.0) | 6.1 (3.1, 11.9) |
| | *HPV 18* | *14/203* | *8.2 (4.5, 13.4)* | *90.9 (89.6, 92.1)* | *6.9 (3.8, 11.3)* | 7.7 (6.5, 8.9) | *0.9 (0.5, 1.5)* |
| | *HPV 16/18* | *106/795* | *62.0 (54.3, 69.3)* | *66.9 (64.9, 69.0)* | *13.3 (11.0, 15.9)* | 4.5 (3.5, 5.6) | *3.0 (2.2, 4.0)* |
| CIN3+ ($N = 80$) | HPV 33 | 8/67 | 10.0 (4.4, 18.8) | 97.3 (96.5, 97.9) | 11.9 (5.3, 22.2) | 3.3 (2.6, 4.1) | 3.6 (1.8, 7.2) |
| | HPV 16 | 53/632 | 66.3 (54.8, 76.4) | 73.4 (71.5, 75.2) | 8.4 (6.3, 10.8) | 1.7 (1.1, 2.4) | 5.0 (3.2, 7.9) |
| | HPV 35 | 3/50 | 3.8 (0.8, 10.6) | 97.8 (97.1, 98.4) | 6.0 (1.3, 16.5) | 3.5 (2.8, 4.3) | 1.7 (0.6, 5.2) |
| | Cluster A | 63/727 | 78.8 (68.2, 87.1) | 69.5 (67.5, 71.4) | 8.7 (6.7, 11.0) | 1.1 (0.6, 1.8) | 7.8 (4.6, 13.2) |
| | Cluster A or B | 77/1341 | 96.3 (89.4, 99.2) | 41.9 (39.8, 44.0) | 5.7 (4.6, 7.1) | 0.3 (0.1, 1.0) | 17.5 (5.5, 55.3) |
| | Cluster A or B or C | 77/1685 | 96.3 (89.4, 99.2) | 26.1 (24.2, 28.0) | 4.6 (3.6, 5.7) | 0.5 (0.1, 1.5) | 8.7 (2.8, 27.5) |
| | *HPV 18* | *4/203* | *5.0 (1.4, 12.3)* | *90.9 (89.6, 92.0)* | *2.0 (0.5, 5.0)* | 3.7 (2.9, 4.6) | *0.5 (0.2, 1.4)* |
| | *HPV 16/18* | *57/795* | *71.3 (60.0, 80.0)* | *66.1 (64.0, 68.1)* | *7.2 (5.5, 9.2)* | 1.6 (1.0, 2.4) | *4.6 (2.8, 7.3)* |

[1]Lesions detected at baseline within 1 year of enrolment.
[2]$n$ is the number of CIN2+ or CIN3+ cases; $N$ is the number of women positive for the HPV type.
[3]Risk of carrying CIN2+ (or CIN3+) among HC2 positive women who were positive for the relevant genotypes divided for the same risk in HC2 positive women who were negative for that genotypes.

**Table 4.** Accuracy of individual genotypes and clusters for HC2 positive women[1]

| Endpoint | HPV types | $n/N$[2] | Sensitivity | Specificity | PPV | 1-NPV | Relative risk[3] |
|---|---|---|---|---|---|---|---|
| CIN2+ ($N = 239$) | HPV 33 | 17/67 | 7.1 (4.2, 11.1) | 97.5 (96.7, 98.2) | 25.4 (15.5, 37.5) | 10.1 (8.9, 11.5) | 2.5 (1.6, 3.8) |
| | HPV 35 | 12/50 | 5.0 (2.6, 8.6) | 98.1 (97.4, 98.7) | 24.0 (13.1, 38.2) | 10.3 (9.1, 11.6) | 2.3 (1.4, 3.8) |
| | HPV 16 | 124/632 | 51.9 (45.3, 58.4) | 74.8 (72.8, 76.7) | 19.6 (16.6, 22.9) | 7.1 (5.9, 8.4) | 2.8 (2.2, 3.5) |
| | Cluster A | 150/727 | 62.8 (56.3, 68.9) | 71.4 (69.4, 73.3) | 20.6 (17.7, 23.8) | 5.8 (4.7, 7.1) | 3.5 (2.7, 4.5) |
| | Cluster A or B | 220/1493 | 92.1 (87.9, 95.1) | 36.9 (34.7, 39.0) | 14.7 (13.0, 16.6) | 2.5 (1.5, 3.9) | 5.9 (3.7, 9.4) |
| | Cluster A or B or C | 226/1685 | 94.6 (90.9, 97.1) | 27.6 (25.7, 29.6) | 13.4 (11.8, 15.1) | 2.3 (1.2, 3.9) | 5.9 (3.4, 10.2) |
| | *HPV 18* | *19/203* | *7.9 (4.9, 12.1)* | *90.9 (89.5, 92.1)* | *9.4 (5.7, 14.2)* | *10.7 (9.4, 12.1)* | *0.9 (0.6, 1.4)* |
| | *HPV 16/18* | *139/795* | *58.2 (51.6, 64.5)* | *67.5 (65.4, 69.5)* | *17.5 (14.9, 20.3)* | *6.8 (5.6, 8.3)* | *2.6 (2.0, 3.3)* |
| CIN3+ ($N = 113$) | HPV 33 | 8/67 | 7.1 (3.1, 13.5) | 97.2 (96.5, 97.9) | 11.9 (5.3, 22.2) | 4.8 (3.9, 5.8) | 2.5 (1.3, 4.9) |
| | HPV 16 | 68/632 | 60.2 (50.5, 69.3) | 73.7 (71.7, 75.5) | 10.8 (8.5, 13.4) | 2.8 (2.0, 3.7) | 3.8 (2.6, 5.5) |
| | HPV 35 | 5/50 | 4.4 (1.5, 10.0) | 97.9 (97.2, 98.5) | 10.0 (3.3, 21.8) | 4.9 (4.0, 5.9) | 2.0 (0.9, 4.7) |
| | Cluster A | 80/727 | 70.8 (61.5, 79.0) | 69.8 (67.8, 71.7) | 11.0 (8.8, 13.5) | 2.2 (1.5, 3.0) | 5.0 (3.4, 7.4) |
| | Cluster A or B | 107/1435 | 94.7 (88.8, 98.0) | 38.0 (35.9, 40.1) | 7.5 (6.2, 8.9) | 0.7 (0.3, 1.6) | 10.7 (4.7, 24.2) |
| | Cluster A or B or C | 109/1685 | 96.5 (91.2, 99.0) | 26.4 (24.6, 28.3) | 6.5 (5.3, 7.8) | 0.7 (0.2, 1.8) | 9.2 (3.4, 24.8) |
| | *HPV 18* | *7/203* | *6.2 (2.5, 12.3)* | *90.8 (89.5, 92.0)* | *3.4 (1.4, 7.0)* | *5.2 (4.2, 6.2)* | *0.7 (0.3, 1.4)* |
| | *HPV 16/18* | *75/795* | *66.4 (56.9, 75.0)* | *66.4 (64.3, 68.4)* | *9.4 (7.5, 11.7)* | *2.6 (1.8, 3.6)* | *3.6 (2.5, 5.3)* |

[1]Lesions detected at baseline or during follow-up analysis Within 3 years of recruitment.
[2]$n$ is the number of CIN2+ or CIN3+ cases; $N$ is the number of cases positive for the HPV type.
[3]Risk of carrying or developing CIN2+ (or CIN3+) among HC2 positive women who were positive for the relevant genotypes divided for the same risk in HC2 positive women who were negative for that genotypes.

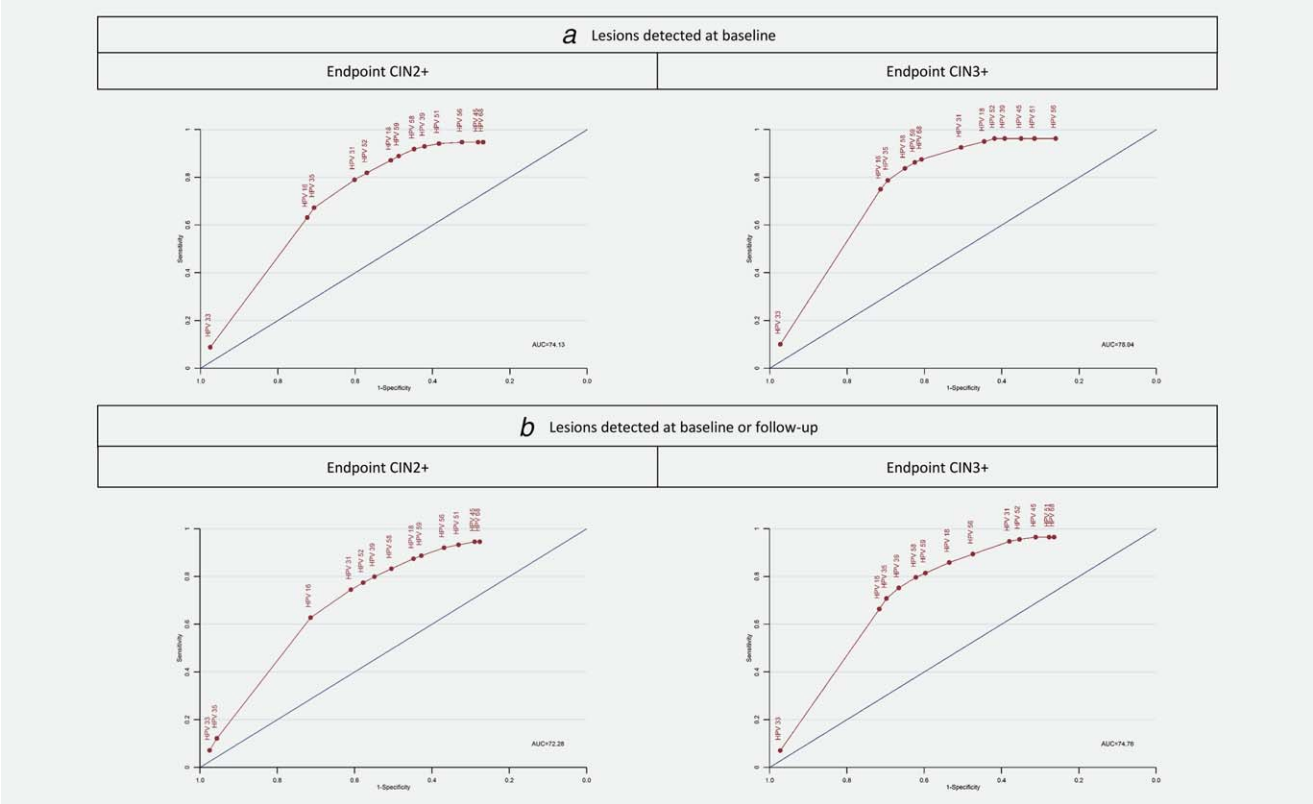


**Figure 1.** Hierarchical ROC Curves for genotyping among HPV-positive women. Hierarchical ranking of HPV types was obtained by iteratively determining the subsequent genotype with the highest PPV excluding cases with multiple infections with higher ranking types. ROC curves show cumulative sensitivity and specificity accounting for the genotype hierarchy. [Color figure can be viewed at wileyonlinelibrary.com]

5.8% (95% CI 4.7–7.1) and decreased to 2.3% (95% CI 1.2–3.9) for types included in all three clusters. The PPV of CIN2+ was 20.6% (95% CI 17.7–23.8) if positive for HPV types in cluster A, and 14.7% (95% CI 13.0–16.6) and 13.4% (95% CI 11.8–15.1) cumulatively adding cluster B and C respectively.

The corresponding ROC curves for cumulative sensitivity and specificity after adjusting for type hierarchy are shown in Figure 1. The areas under the ROC curves were 78.0% for CIN3+ and 74.1% for CIN2+ when considering lesions detected at baseline (panel A), and slightly decreased to 74.8% for CIN3+ and 72.3% for CIN2+ when considering all lesions detected at baseline or follow-up (panel B).

The relative risk of carrying a CIN3+ reached a maximum (17.5%) for women positive to the genotypes included in clusters A and B *versus* those negatives to the same types and then decreased to 8.7% when comparing the women positives to the genotypes included in clusters A, B and C to those negative. The same pattern was present for carrying CIN2+ and for longitudinal data. In any case, the single genotype associated to the highest relative risk (RR) was always HPV16.

The PPP plots, relative to positivity for HPV16, HPV16 or 18, cluster A, and cluster A + B are reported in Figure 2. A post-test risk higher than 10% was obtained by positivity for HPV16 or cluster A, while the adding of other types slightly lowered this level.

## Discussion

We have analyzed the cross-sectional and longitudinal association of high-risk HPV genotypes with CIN2+ and CIN3+ among women who tested positive for HC2 in a large cohort of Italian women enrolled in the NTCC trial. The hierarchical analysis for cross-sectional and longitudinal risk of developing high-grade lesions allowed grouping high-risk HPV types into three clusters. Although ranking differences were observed in relation to different endpoints (lesion grade and time of detection), results were highly consistent. Cluster A, characterized by the highest PPVs, included, besides HPV16 (an expected observation), HPV33 and HPV35, while HPV18 was ranked lower in cluster B and HPV45 in cluster C.

Both HPV33 and 35 had a low prevalence in the study population. HPV33 ranked first based on PPV thus showing strong association with present and future CIN2+ and CIN3+ risk. This result is consistent with other recent reports which found a strong association between HPV33 and high-grade CIN despite a much weaker association with invasive cancer.[8,24–27] Conversely, the importance of HPV35 based on PPV was more unexpected even though a previous analysis within the future study[28] reported a high probability of transition from HPV35 incident infection to CIN2 and CIN3 lesions after 3 years of follow-up. In other studies, the association between HPV35 and high-grade CIN varied

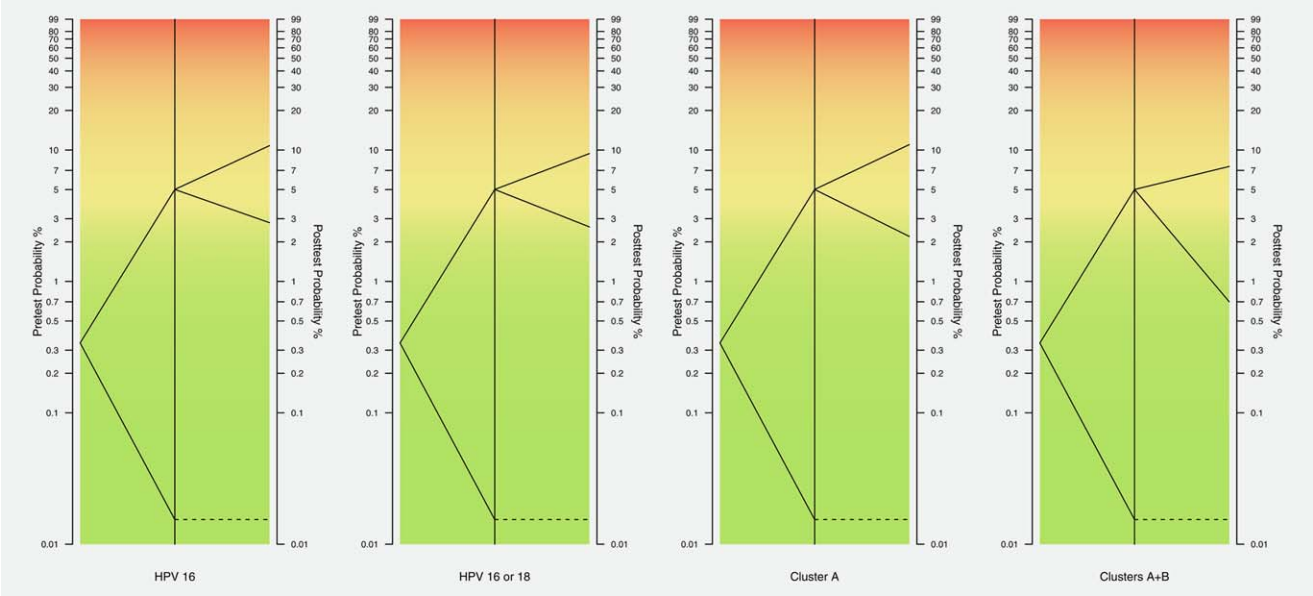


**Figure 2.** Pre-test and post-test probability plots for screening by HC2 and triage by selected groups of genotypes. The plotted probabilities are of carrying CIN2+. For groups of genotypes, the post-positive-test and post-negative-test probabilities were the corresponding PPV and 1-NPV, respectively. The post-negative-HPV probability was computed including all the lesions detected up to the following screening round within NTCC (up to 3 years).

remarkably between different geographical areas.[8,29] In a recent analysis on viral load and CIN risk,[30] HPV35 was one of the four non HPV16/18 types (with HPV31, 52 and 58) whose viral load at baseline was significantly associated with a subsequent increase in CIN2/3 risk.

On the other hand, HPV18 and 45 showed a low PPV for CIN2/3+ despite being responsible for a considerable proportion of invasive cancers.[31,32] This discrepancy, already observed in many studies having high-grade CIN as the endpoint,[6,10,33,34] could result from a different natural history of cell transformation induced by HPV18 and 45. In fact, these genotypes are over-represented in adenocarcinomas and early onset cancers[35–37] and are rarely prevented through cytological screening.[2,38,39] It is, however, not clear if for these genotypes the co-existing high risk of developing invasive cancer and low risk of having a pre-cancerous lesion detected depends on a high rate of progression of such lesions to invasion or on the difficulty in detecting them by colposcopy. In any case, these two possible mechanisms result in a too short detectable sojourn time of pre-invasive lesions. This makes cytological screening relatively ineffective in preventing invasive cancers caused by these genotypes.[40]

The risk ratios between HPV16 and the remaining hrHPV types observed in cohort studies evaluating the long-term CIN3+ risk were 4.5 at 12 years in Kjær *et al.*[10] and 5.8 at 10 years in Khan *et al.*[9] Our corresponding RRs comparing hierarchically HPV16 to the remaining HC2 positive women were quite similar (5.0 [95%CI 3.2–7.9] and 3.8 [95%CI 2.6–5.5]). Some characteristics of our study, such as higher women's age at enrolment (median 38 years *vs.* 34 and 28 years) and shorter length of follow-up can partly account for these differences. CIN3 and invasive cervical cancers associated with HPV16 are diagnosed at an earlier age than the corresponding lesions associated with other HPV types.[11]

Concerning the use as a triage test, the sensitivity of HPV16 does not seem sufficient (66.3% for CIN3+ cross-sectional) to be used alone as a triage test in high-income countries. Adding HPV33 and 35 could be reasonable but it must be taken into account that high-grade CIN caused by these types could have a lower progression rate to invasion. In cluster A, the cross-sectional sensitivity for CIN3+ reached 78.8% and the risk of CIN3+ in HC2 positive women without these types was 1.1%. By comparison, the cross-sectional sensitivity for CIN3+ was 88.1% with ASC-US+ cytology with knowledge of HPV infection (corresponding 1-NPV: 0.6%)[41] and 91% with p16.[42] Adding other genotypes would increase sensitivity but at the cost of a strong loss in specificity. HPV18 and 45 represent a possible exception if the corresponding CIN had high probability of progression to invasion, which is unknown. The sensitivity and specificity of partial HPV16/18 genotyping, available for some clinically validated screening assays, was lower than that of HPV16 genotyping alone.

The areas under the ROC curves for cumulative sensitivity and specificity gave values around and above 75%, indicating good discriminating capacity as a triage test. Nonetheless, although this evaluation allows to compare two tests with very high accuracy, other factors must be taken into account, and it is considered a theoretical measure. On the other hand, the PPP plots, constructed to estimate the clinical use of different strategies of HPV genotyping, highlight that positivity for HPV16 or cluster A types (i.e. 33, 16 and 35) confer a risk >10%, while negativity for cluster A + B types confer a risk <1%. Indeed, while immediate colposcopy is generally

warranted for a risk >10%, different and country-specific decision thresholds do exist for risk levels in the 1–10% range.[43]

Combining genotyping with other triage methods (*e.g.*, cytology) seems more promising than increasing the number of "positive" genotypes[44] but this will be examined elsewhere.

This study has strengths and limitations. Our results are derived from a large randomized trial nested in an organized population-based screening program, where over 70% of eligible women were enrolled, suggesting good applicability to routine activity. All HPV-positive women were referred to colposcopy, and both participation to and completeness of follow-up were high, so minimizing verification bias. Genotyping was blind to cytology and histology and its completeness within the areas/phases in which it was done was high and plausibly unselected. On the other hand, despite the large size, the precision of estimates for some less frequent HPV types is low and random variation could explain the differences between our observations and those obtained in other studies.[8,44] Previous screening intensity can account for differences in PPV compared to other studies[24] and local variations in type distribution can result in differences in sensitivity and specificity. Indeed, type distribution changes between continents[45] but also between areas in the same country.[21] Differences with other studies can also result from different analytical sensitivity and specificity of different genotyping assays for different genotypes. For this reason, our result may not reflect exactly the PPV of typing, if we start from a different HPV DNA primary test (including starting directly with GP5+/GP6+ primed PCR). In fact some 10.7% of HC2 positive women were negative for GP5+/GP6+ primed PCR and plausibly another proportion negative to HC2 would have been positive to GP5+/GP6+. Nonetheless, cross-sectional PPV and sensitivity for CIN3+ of HPV16 (8.4% and 66.3%, respectively) was consistent with what observed in the Addressing the Need for Advanced HPV Diagnostics (ATHENA) study[34] (18.3% and 50.4%, respectively) and with a recent Italian study (18.8% and 55.6%, respectively),[46] both using the COBAS 4800 HPV test (Roche) with partial genotyping.

In conclusion, we studied the cross-sectional and short-term longitudinal association of high-risk HPV genotypes with high grade CIN on a large unselected population and found HPV 33, 16 and 35 to be the most strongly associated. Nevertheless, in this study the gradient was not sufficient to use genotyping alone as a method to triage HPV-positive women, and combination with other tests is needed, taking also into account that high-grade CIN caused by different genotypes could have different probabilities of progression to invasion.



## Acknowledgements

This study was supported by Ministero della Salute (Progetto Speciale "Valutazione di nuove tecnologie per lo screening del cervicocarcinoma – follow-up" and L 138–2004 progetto "NTCC—Nuove tecnologie per lo screening del cervicocarcinoma"), by FP7 Health (CoHeahr project, FP7 grant agreement no. HEALTH-F3–2013–603019) and by the Associazione Italiana per la Ricerca sul Cancro (AIRC, Project IG14119). Research funding was also obtained from Qiagen, BD, Abbott, Hologic, Trovagene, Oncohealth, Genera and Cepheid (to J.C.). P.G.R. as PI of an independent study funded by the Italian Ministry of Health, data owner, conducted negotiations with Roche diagnostics, Hologic, Qiagen, and Abbott to obtain reagents at reduced price or for free.

## References

1. Arbyn M, Ronco G, Anttila A, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. *Vaccine* 2012;30:F88–99.
2. Ronco G, Dillner J, Elfström M, International HPV screening working group, et al. Efficacy of HPV-based screening for preventing invasive cervical cancer: follow-up of European randomised controlled trials. *Lancet* 2014;383:524–32.
3. Waller J, McCaffery K, Kitchener H, et al. Women's experiences of repeated HPV testing in the context of cervical cancer screening: a qualitative study. *Psychooncology* 2007;16:196–204.
4. Ronco G, Franceschi S, Segnan N. HPV16 and HPV18 genotyping in cervical cancer screening (invited comment). *Lancet Oncol* 2011;12:831–2.
5. Bouvard V, Baan R, Straif K, on behalf of the WHO IARC in Cancer Monograph working group, et al. A review of human carcinogens – Part B: biological agents. *Lancet Oncol.* 2009;10:321–2.
6. Castle PE, Aslam S, Behrens C. Cervical precancer and cancer risk by human papillomavirus status and cytologic interpretation: implications for risk management. *Cancer Epidemiol Biomarkers Prev* 2016;25:1595–9.
7. Rodriguez AC, Schiffman M, Herrero R, et al. Longitudinal study of human papillomavirus persistence and cervical intraepithelial neoplasia grade 2/3: critical role of duration of infection. *J Natl Cancer Inst* 2010;102:315–24.
8. Guan P, Howell-Jones R, Li N, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *Int J Cancer* 2012;131:2349–59.
9. Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J Natl Cancer Inst* 2005;97:1072–9.
10. Kjær SK, Frederiksen K, Munk C, et al. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst* 2010;102:1478–88.
11. Tjalma WA, Fiander A, Reich O, for the HERACLES/SCALE Study Group, et al. Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. *Int J Cancer* 2013;132:854–67.
12. Saslow D, Solomon D, Lawson HW, et al. American Cancer Society; American Society for Colposcopy and Cervical Pathology; American Society for Clinical Pathology. American Cancer Society, American Society for Colposcopy and Cervical Pthology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *Am J Clin Pathol* 2012;137:516–42.
13. Moyer VA. U.S. Preventive Services Task Force. Screening for cervical cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2012;156:880–91.
14. Ronco G, Segnan N, Giorgi-Rossi P, the NTCC Working Group, et al. Human Papillomavirus testing and liquid-based cytology: results at recruitment from the New Technologies for Cervical Cancer randomized controlled trial. *J Natl Cancer Inst* 2006;98:765–74.
15. Ronco G, Giorgi-Rossi P, Carozzi F, the NTCC Working Group, et al. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol* 2006;7:547–55.
16. Ronco G, Giorgi-Rossi P, Carozzi F, the NTCC Working Group, et al. Results at recruitment from a randomized controlled trial comparing human papillomavirus testing alone to conventional cytology as the primary cervical cancer screening test. *J Natl Cancer Inst* 2008;100:492–501.

17. Ronco G, Giorgi-Rossi P, Carozzi F, et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. *Lancet Oncol* 2010;11:249–57.
18. Jacobs MV, Roda Husman AM, van den Brule AJ, et al. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. *J Clin Microbiol* 1995;33:901–5.
19. van den Brule AJ, Pol R, Fransen-Daalmeijer N, et al. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol* 2002;40:779–87.
20. Gillio-Tos A, De Marco L, Carozzi FM, the New Technologies for Cervical Cancer Screening (NTCC) working group, et al. Clinical impact of the analytical specificity of hybrid capture 2: data from the NTCC study. *J Clin Microbiol* 2013;51: 2901–7.
21. Carozzi F, De Marco L, Gillio-Tos A, NTCC Working Group, et al. Age and geographic variability of human papillomavirus high-risk genotype distribution in a large unvaccinated population and of vaccination impact on HPV prevalence. *J Clin Virol* 2014;60:257–63.
22. Dalla Palma P, Giorgi Rossi P, Collina G, and the NTCC Pathology Group, et al. The reproducibility of CIN diagnoses among different pathologists: data from histology reviews from a multicentre randomized study. Am. *J Clin Pathol* 2009;132:125–32.
23. Dalla Palma P, Giorgi-Rossi P, Collina G, and the NTCC Pathology Group, et al. The risk of false-positive histology according to the reason for colposcopy referral in cervical cancer screening: a blind revision of all histological lesions found in the NTCC trial. *Am J Clin Pathol* 2008;129:75–80.
24. Cuzick J, Ho L, Terry G, et al. Individual detection of 14 high risk human papilloma virus genotypes by the PapType test for the prediction of high grade cervical lesions. *J ClinVirol* 2014;60: 44–9.
25. Smelov V, Elfstrom KM, Johansson ALV, et al. Long-term HPV type-specific risks of high-grade cervical intraepithelial lesions: a 14-year follow-up of a randomized primary HPV screening trial. *Int J Cancer* 2015;136:1171–80.
26. Schiffman M, Hyun N, Raine-Bennett TR, et al. A cohort study of cervical screening using partial HPV typing and cytology triage. *Int J Cancer* 2016;139:2606–15.
27. Jaisamrarn U, Castellsagué X, Garland SM, HPV PATRICIA Study Group, et al. Natural history of progression of HPV infection to cervical lesion or clearance: analysis of the control arm of the large, randomized PATRICIA study. *PLoS ONE* 2013;8: e79260.
28. Insinga RP, Perez G, Wheeler CM, for the FUTURE I Investigators, et al. Incident cervical HPV infections in young women: transition probabilities for CIN and infection clearance. *Cancer Epidemiol Biomarkers Prev* 2011;20:287–96.
29. Kahng J, Kim EH, Kim HG, et al. Development of a cervical cancer progress prediction tool for human papillomavirus-positive Koreans: a support vector machine-based approach. *J Int Med Res* 2015;43:518–25.
30. Xi LF, Schiffman M, Ke Y, et al. Type-dependent association between risk of cervical intraepithelial neoplasia and viral load of oncogenic human papillomavirus types other than types 16 and 18. *Int J Cancer* 2017;140:1747–56.
31. de Sanjose S, Quint WGV, Alemany L, Retrospective International Survey and HPV Time Trends Study Group, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol* 2010;11:1048–56.
32. Li N, Franceschi S, Howell-Jones R, et al. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer* 2011;128:927–35.
33. Monsonego J, Cox JT, Behrens C, et al. Prevalence of high-risk human papillomavirus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol Oncol* 2015;137: 47–54.
34. Castle PE, Stoler MH, Wright TC, Jr, et al. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *Lancet Oncol* 2011;12:880–90.
35. Castellsagué X, Diaz M, de Sanjosé S, et al. Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. *J Natl Cancer Inst* 2006;98:303–15.
36. Baalbergen A, Smedts F, Ewing P, et al. HPV-type has no impact on survival of patients with adenocarcinoma of the uterine cervix. *Gynecol Oncol* 2013;128:530–4.
37. Clifford G, Franceschi S. Members of the human papillomavirus type 18 family (alpha-7 species) share a common association with adenocarcinoma of the cervix. *Int J Cancer.* 2007;122:1684–5.
38. Castanon A, Landy R, Sasieni PD. Is cervical screening preventing adenocarcinoma and adenosquamous carcinoma of the cervix?. *Int J Cancer* 2016;139:1040–5.
39. Zucchetto A, Ronco G, Giorgi Rossi P, IMPATTO CERVICE Working Group, et al. Screening patterns within organized programs and survival of Italian women with invasive cervical cancer. *PrevMed* 2013;57:220–6.
40. Sasieni PD, Castanon A, Cuzick J. Screening and adenocarcinoma of the cervix. *Int J Cancer* 2009; 125:525–9.
41. Bergeron C, Giorgi Rossi P, Cas F, et al. Informed cytology for triaging HPV-positive women: substudy nested in the NTCC randomized controlled trial. *J Natl Cancer Inst* 2015;107: pii.
42. Carozzi F, Confortini M, Dalla Palma P, the New Technologies for Cervical Cancer screening (NTCC) working group, et al. Use of p16-INK4A overexpression to increase the specificity of human papillomavirus testing: a nested substudy of the NTCC randomised controlled trial. *Lancet Oncol* 2008;9:937–45. and
43. Arbyn M, Xu L, Verdoodt F, et al. Genotyping for Human Papillomavirus types 16 and 18 in women with minor cervical lesions. A systematic review and meta-analysis. *Ann Intern Med* 2017; 166:118–27.
44. Schiffman M, Khan MJ, Solomon D, PEG Group; ALTS Group, et al. A study of the impact of adding HPV types to Cervical Screening and triage tests. *J Natl Cancer Inst* 2005;97:147–50.
45. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cancer. *Lancet* 2007; 370:890–907.
46. Gustinucci D, Giorgi Rossi P, Cesarini E, et al. Use of cytology, E6/E7 mRNA, and $p16^{INK4A}$-Ki-67 to define the management of Human Papillomavirus (HPV)-positive women in cervical cancer screening. *Am J Clin Pathol* 2016;145:35–45.