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Age and geographic variability of human papillomavirus high-risk genotype distribution in a large unvaccinated population and of vaccination impact on HPV prevalence

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ABSTRACT

Background: The prevalence of infections with human papillomavirus (HPV) specific genotypes differs by age and areas. Knowledge of these differences will help predicting how prophylactic HPV vaccination and screening program could best be integrated.
Objectives: To investigate variations in the HPV distribution between areas and ages in Italy and the impact of vaccination on HPV prevalence.
Study design: 37,367 women aged 25–60 years who attended cervical screening in eight different areas in Northern and Central Italy were tested for HPV infection with the high-risk hybrid capture (hr-HC2) assay. hr-HC2 positive samples were genotyped by an intensive integrated strategy.
Results: hr-HPV types were detected in 79.1% of HC2 positive women. HPV16 was the most frequent type, followed by HPV31, HPV18 and HPV56. A statistically significant variability in HPV type distribution between centres (overall $\chi^2_{84df} = 195.86$ $p < 0.001$) was observed. No significant overall difference in the HPV type distribution was observed in the age groups 25–34, 35–44 and 45–60 years. Considering cross-protection, overall 57.6% (95%CI 56.0–59.3) of all infections by hr-HPV types was preventable by vaccination with the bivalent vaccine and 49% (95%CI 46.9–51.1) with the quadrivalent vaccine. The variability between centres was statistically significant with both bivalent ($\chi^2_{7df} = 43.8$, $p < 0.0001$) and quadrivalent vaccine ($\chi^2_{7df} = 32.9$, $p < 0.0001$).
Conclusions: We observed differences in HPV genotype distribution according to centres but not to age. Results suggest that the higher proportion of HPV16/18 related high grade CIN in younger women could be the result of faster progression and not of earlier infection by these types.

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; CIN2+, cervical intraepithelial lesion grade 2 or more severe; CO, mean of three concurrently tested controls; hgCIN, high-grade CIN; HPV, human papillomavirus; hr-HC2, high-risk hybrid capture 2; HSIL, high grade squamous intraepithelial lesion; HSIL+, high grade squamous intraepithelial lesion or more severe; LSIL, low grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesions or malignancy; NTCC, new technologies for cervical cancer screening; RCT, tandomized controlled trial; RFLP, restriction fragment length polymorphism; RLB, reverse line blot; RLU, relative light units; STM, specimen transport medium; WHO, World Health Organization.

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1. Background

Two new approaches for the prevention of cervical cancer have emerged over the past decade: HPV16 and 18 vaccination and screening by HPV testing [1]. In addition, recent results provided evidence that HPV16/18 vaccines also confer cross-protection against persistent infections by other high-risk (hr) HPVs [2–5].

Screening based on DNA testing for carcinogenic HPV types allows earlier detection of clinically relevant precancerous lesions and higher efficacy than cytology-based screening [6–9].

Knowledge of the genotype-specific prevalence of HPV infection by age and area is important to predict how these two approaches might influence cervical cancer prevention and how prophylactic HPV vaccination and screening could best be integrated.

A large number of studies considered the age-specific prevalence of HPV infection in different world areas [10,11]. They studied the age profiles of the overall prevalence of HPV infection, showing relevant variability in different countries [12,13]. However, little is known on if and how the HPV type changes by age in women without cervical intraepithelial lesion (CIN) [14–17]. In addition, it has not been widely studied if the geographical variability of HPV genotypes remains relevant also between close areas, within the same country.

2. Objectives

We used the data from a large population-based randomized controlled trial, the new technologies for cervical cancer screening (NTCC) study, to investigate variations in the type distribution by cervical cytology and between areas and ages in Italy. Moreover, we evaluated the effect of the two HPV vaccines on type distribution.

3. Study design

Population enrolled and protocols applied during the NTCC study have been previously described in detail [18–20]. Briefly, after written informed consent, women aged 25–60 years attending for a new round of routine cervical-cancer screening in nine different Italian organized screening programs were enrolled in the NTCC trial and randomly assigned to two study groups: conventional (conventional cytology) and experimental (hr-HPV testing with liquid-based cytology, or alone respectively during two preplanned study phases).

3.1. Sample collection and storage

Cervical scrape samples were collected in ThinPrep® vials (Cytyc Corporation, Marlborough, USA) during phase 1 and in specimen transport medium (STM, DNAPAP Cervical Sampler, Qiagen, Gaithersburg, USA) during phase 2. Residual material for genotyping from HC2 positive women was stored in four centres (Turin, Padua, Trento and Florence) during both study phases and in four other centres (Bologna, Imola, Ravenna and Viterbo) just during phase 2.

3.2. HPV screening test and genotyping

Testing for the DNA of high-risk HPV types was done by Hybrid Capture 2 (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68), with the 1 RLU/CO producer-recommended cut-off. HPV genotyping was performed in Florence, Padua, Turin and Trento. DNA was extracted using the QIAamp DNA Mini kit (Qiagen) with a double final elution to optimize recovery of DNA. A PCR assay [21] employing GP5+/GP6+ consensus primers (Digene HPV Genotyping RH kit, Qiagen) was performed [16] followed by a reverse line blot (RLB) for detection of 12 high-risk HPV types (Group 1: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59), one probably carcinogenic HPV type (Group 2A: 68), and five possibly carcinogenic HPV types (Group 2B: 26, 53, 66, 73 and 82) [22]. Henceforth, the genotypes belonging to Groups 1 and 2A, i.e. those targeted by the HC2 test, will be defined as hr-HPV types.

PCR biotinylated products were denaturated and hybridized at 50 °C with type-specific oligonucleotide probes immobilized on nitrocellulose membrane strips. The hybrids were detected with alkaline phosphatase–streptavidin conjugate and substrate (5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium). After washing and drying, the strips were analysed visually from an interpretation grid supplied in the kit. GP5+/GP6+ PCR-negative and RLB-negative samples were amplified for the β-globin gene using GH20-PC04 primers [23].

To overcome negative results due to low copy number of HPV DNA, HPV-negative/β-globin positive samples were further analysed by nested PCR using MY09/MY11 and GP5+/6+ primer sets [24] followed by RLB hybridization (Consensus High Risk HPV Genotyping kit), as previously described. The GP5+/6+ PCR-positive and RLB-negative samples were considered un-typable with employed system and submitted to further analytical procedures to identify the exact HPV genotype present: restriction fragment length polymorphism (RFLP) analysis of MY09/MY11 amplimers [25] or direct sequencing of GP or MY amplified products [16].

3.3. Genotyping quality control among NTCC laboratories

Evaluation of the GP5+/6+ Digene RLB kit sensitivity for HPV18 and HPV16 was assessed in each laboratory by using the international standards for HPV16 (NIBSC 06/202) and for HPV18 (NIBSC 06/206) in serial dilutions. All four laboratories obtained an analytical sensitivity for both HPV16 and HPV18 of 50 genome equivalents/5 μl. Overall, 66 samples previously typed with methods routinely used in the laboratories were tested; fully concordant results were obtained in 60 samples with a 90% agreement.

Moreover in 2010 and in 2011, three of the NTCC laboratories (Florence, Padua and Torino) participated to the WHO HPV LabNet 2010 and 2011 Proficiency Studies as external validation of quality, testing the provided panel of samples with the Digene HPV Genotyping RH kit. The sensitivity limit for all three laboratories was 50 copies/5 μl for HPV16, 18, 31, 33, 35, 45, 56, 66 and 500 copies/5 μl for HPV39, 51, 52, 58, 68a, 68ME and 59.

3.4. Statistical analysis

The main endpoint was the distribution of genotypes among the total number of infections detected. Only infections from the genotypes targeted by HC2 were considered. Analyses were conducted both including and excluding the infections from women with cervical intraepithelial neoplasia grade 2 or 3 or invasive cancer (CIN2+). For women recruited during the phase 1, who had cytology, the type distribution is also reported by cytology results (negative for intraepithelial lesions or malignancy [NILM], atypical squamous cells of undetermined significance [ASC-US], low grade squamous intraepithelial lesion [LSIL], high grade squamous intraepithelial lesion or more severe [HSIL+]).

The reciprocally adjusted effect of age (linear) and centre on the proportion of women with multiple infections among those infected was estimated by unconditional logistic regression.

The overall variability in genotype distribution between centres was tested by χ^2 test. Cell χ^2s were studied to identify the most relevant deviations. Given the high number of cells, only cell χ^2s > 6.63 (equivalent to $p < 0.01$) were considered as relevant. In order to evaluate if differences between centres were explained by