

## ORIGINAL ARTICLE

# Cervical Human Papillomavirus genotypes in HIV-infected women: a cross-sectional analysis of the VALHIDATE study

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## Keywords

HIV-infected women • HPV types • Cervical lesions • Molecular Epidemiology

## Summary

**Introduction.** Primary-prevention by prophylactic vaccination against HPV-related cancers and HPV-based screening programs are based on HPV-type distribution in immunocompetent individuals. HIV-infected women are at high risk of invasive HPV-disease sustained by a broader range of HPV-types and have higher multi-type infection rates than immunocompetent hosts.

**Methods.** This is a cross-sectional analysis of High Risk HPV (HR HPV) type distribution in 805 HIV+ women (HIW) compared with a control group of 1402 immunocompetent HIV- women (SPW) enrolled in the VALHIDATE study in order to define HPV type-specific distribution according to cytology.

**Results.** HIW had a 3.8, 3.6, and 2.7 times higher risk of atypical squamous cells of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) than SPW respectively.

HPV-DNA prevalence was 28.4% in HIW and 11.81% in SPW ( $p < 0.0001$ ). The prevalence of infection increased from normal cytology to HSIL both in HIW (from 21.45% to 90.91%) and SPW (from 9.54% to 75%). The OR for women with normal cytology of having a positive HPV-DNA test result of was 2.6 times higher in HIW than in SPW. The cumulative prevalence of HPV-16/18 in HSIL is much lower in HIW ( $36.4 \pm 28.4$ ) than SPW ( $62.5 \pm 33.5$ ).

**Conclusions.** A higher prevalence of infection and broader HPV type distribution were observed in HIV+ women compared to the general population. More than 60% of HSIL lesions of HIW patients are caused by single or multi-type infections from non-HPV16/18 HPVs. The potential 9v-HPV vaccine coverage could be even higher than that expected for the general population given the wide panel of HPV-types observed in the HSIL of HIV+ women.

## Introduction

Thirteen Human Papillomavirus (HPV) genotypes classified as carcinogenic and probably carcinogenic (group 1 and 2A) and six other HPV types with an invasive cervical cancer (ICC)/normal cytology ratio greater than 1.0, classified as possibly carcinogenic genotypes (HPV 26, 30, 67, 69, 73, and 82 – group 2B) are the cause of more than 90% of all ICCs worldwide [1, 2].

Their prevalence varies widely across world regions, but HPV-16 and -18 infections are the most prevalent and carcinogenic all over the world. Apart from the HPV type there are several co-factors that can contribute to invasive evolution [3].

Women affected by HIV/AIDS are at higher risk of invasive disease which is mainly due to the extent of immune-depression [4, 5]. However, the broader range of HPV types sustaining infections and the higher rate of multi-type infections in women living with HIV/AIDS could differently affect HPV type-specific carcinogenic-

ity [6-8]. This is particularly relevant for cervical cancer prevention in immune-compromised hosts: in fact both vaccine primary prevention and HPV-based screening programs are based on the HPV-type distribution in immunocompetent individuals.

Three different HPV vaccines have been approved and licensed by the European Medicines Agency (EMA) and are available in Italy: the 2-valent (Cervarix<sup>®</sup>, GSK biologicals) vaccine, which prevents infections with High Risk HPV (HR HPV) types 16 and 18; the 4-valent vaccine (Gardasil<sup>®</sup>, Merck, Sanofi Pasteur MSD) which also targets Low Risk HPV (LR HPV) types 6 and 11 and the 9-valent vaccine (Gardasil9<sup>®</sup>, Merck, Sanofi Pasteur MSD) which, in addition to the four types of the 4-valent vaccine, also targets five additional HR HPV types (31, 33, 45, 52, and 58). Several studies [9, 10] have indicated that using a 9-valent vaccine could improve the prevention of invasive cervical cancers worldwide from 70% to 90%.

This paper reports a cross-sectional analysis of HPV-type distribution in HIV infected Women (HIW) com-

pared with a control group of immunocompetent HIV-negative women enrolled in the eVALuation and monitoring of HPV infections and related cervical diseases (VALHIDATE) study [11]. The VALHIDATE study [11] was a 5-year (Dec. 2010-Dec.2015) multicenter open prospective cohort study aimed at gaining insight into the molecular epidemiology of HPV infection and cervical diseases in high-risk women in the Lombardy Region, Italy. HIW aged 26-64 years were one of the high-risk cohorts of the study. The control group was composed of women in the same age group attending spontaneous Pap screening-programs (SPW).

The aim of the study was to evaluate HPV type-specific distribution according to cytology among HIV infected women. Moreover, these data will enable us to establish the pre-vaccine type-specific prevalence of HPV-associated diseases in this population in order to evaluate the potential impact of the newly approved 9-valent HPV vaccine.

## Methods

### STUDY DESIGN

With the aim of evaluating the baseline HPV type-specific distribution stratified by the cervical cytological results, HIV-infected women (HIW) were compared with the control group (SPW). HIW and SPW cohorts were recruited consecutively for 12 months from 3 Infectious Diseases Units and 4 Gynecology Units of the four general hospitals located in Lombardy participating in the VALHIDATE study [11]. In particular, HIW were recruited from those followed up for HIV infection and SPW from those attending a spontaneous Pap screening program. Exclusion criteria were: history of histologically proven grade II or higher Cervical Intraepithelial Neoplasia (CIN) requiring treatment, pregnancy at the time of enrollment, inability to provide informed consent.

The protocol enrollment was approved by the Sacco Hospital Ethical Committees (Resolution n174/2010, 9 March 2010) and all participants provided written informed consent.

A total of 828 HIW and 1423 SPW were enrolled in the VALHIDATE study. The consenting women underwent basal co-testing with conventional Pap tests and HPV-DNA testing/genotyping. The cervical brush (Cytobrush Plus MedscandW Medical AB) sample collected at the baseline visit was used to perform the conventional Pap smear and then immersed and stored in a PreservCyt solution (ThinPrep® Pap Test, Hologic Italia Srl) to be analyzed for HPV-DNA and HPV genotyping.

The Pap tests were evaluated according to the 2001 Bethesda System terminology [12] by expert cytopathologists from the participating Centers. The cases were classified according to cytology at baseline evaluation as normal, atypical squamous cells of undetermined significance (ASCUS), low-grade squa-

mous intraepithelial lesions (LSIL) and high-grade SIL (HSIL).

### DNA EXTRACTION, HPV DETECTION AND GENOTYPING

DNA was extracted with a commercial kit (NucliSENS® EasyMAG®, bioMérieux, Lyon, France) and HPV-DNA was detected through PCR amplification of a 450 bp segment of ORF L1 using the degenerate primer pair ELSI-f and ELSI-r in the central reference laboratory of the University of Milan [11, 13]. HPV genotyping was performed on HPV-DNA positive cervical brushes using the commercially available InnoLiPA® HPV Genotyping Extra (Innogenetics N.V., Belgium) method in the microbiology laboratories of the participating Centers. This test allows for the identification of 27 HPV types. All of the HPV-DNA positive cervical samples resulted as non-typeable by the Inno-Lipa test (HPV-X) were subjected to the Restriction Fragment Length Polymorphism (RFLP) type analysis which is capable of identifying all types of the High-Risk clade (HR-clade) and Low-Risk (LR) types of the alpha genus according to the 2011 IARC classification [1, 14].

### STATISTICAL ANALYSIS

HPV-DNA prevalence and type-specific HPV prevalence were expressed as crude proportions with corresponding 95% confidence intervals (95%CI) calculated assuming a normal distribution. The data are presented as the median (interquartile range, IQR) and percentages (with 95%CI) as appropriate. Comparisons between groups were made using the Chi-square test or Fisher's exact test. A P-value less than 0.05 was considered statistically significant (two-tailed test). All of the statistical analyses were performed using GraphPad Prism version 4.02 for Windows, GraphPad Software, San Diego California USA (www.graphpad.com).

## Results

### CYTOLOGICAL RESULTS

Cytological results are provided for 805 HIW (97.2%) and 1402 SPW (98.5%). Women included in the HIW group had an overall 3.7-fold increased risk of ASCUS, a 3.6-fold increased risk of LSIL and a 2.7-fold increased risk of HSIL than those included in the control SPW group (Tab. I).

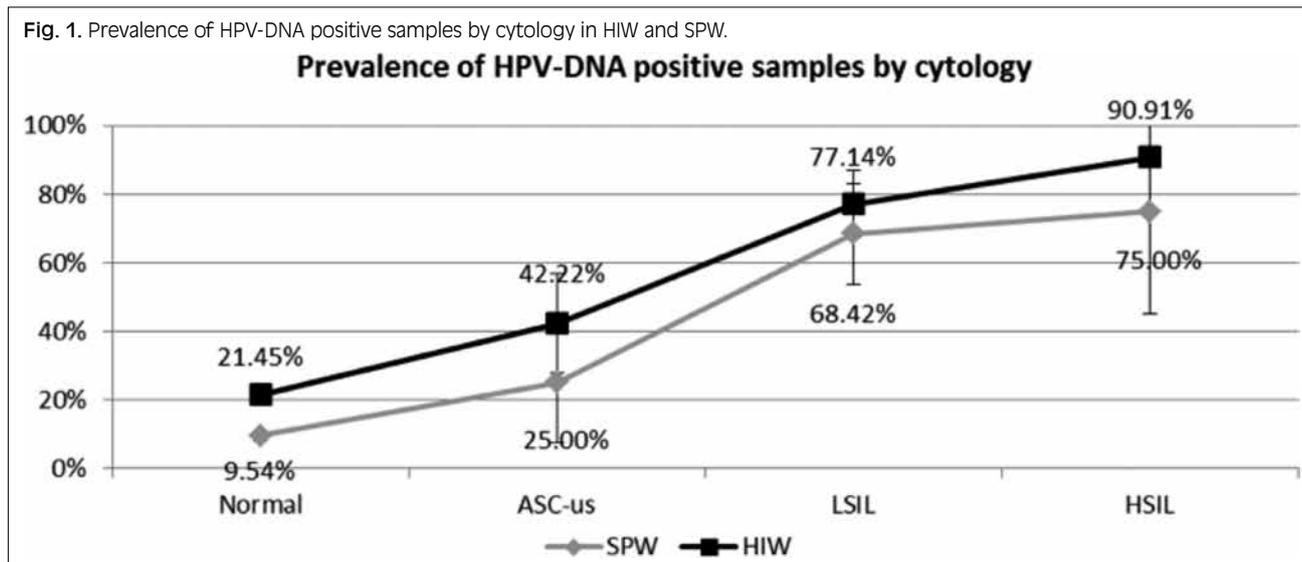
### HUMAN PAPILLOMAVIRUS INFECTION BY CYTOLOGICAL STATUS

HPV-DNA prevalence was 28.4% (95%CI 25.32-31.48) among HIW and 11.81% (95%CI 10.14-13.49) among SPW ( $p < 0.0001$ ). HPV prevalence increased with the progression of the severity of cytological abnormalities from 21.45% to 90.91% in HIW and from 9.54% to

Tab. I. Normal and abnormal cytological results in 805 HIW and 1402 SPW at the baseline evaluation.

Cytology	HIW (n 805)	%	95%CI		SPW (n 1402)	%	95%CI		P value	OR 95%CI
Normal	678	84.22	81.71%	86.74%	1332	95.01	93.87%	96.15%	ref	1
ASCUS	46	5.71	4.11%	7.32%	24	1.71	1.03%	2.39%	< 0.0001	3.765 2.279 to 6.222
LSIL	70	8.70	6.75%	10.64%	38	2.71	1.86%	3.56%	< 0.0001	3.619 2.412 to 5.430
HSIL	11	1.37	0.56%	2.17%	8	0.57	0.18%	0.96%	0.0488	2.701 1.081 to 6.749

Fig. 1. Prevalence of HPV-DNA positive samples by cytology in HIW and SPW.



75% in SPW in normal cytology and HSIL respectively (Fig. 1).

The OR for women with normal cytology of having a positive HPV-DNA was 2.6 times higher in HIW (95%CI 2.0-3.3) than in SPW.

Different HPV-DNA prevalences were found for ASCUS and LSIL, while no differences were observed for HSIL lesions between the two groups (Tab. II).

**HPV TYPING BY CYTOLOGICAL STATUS**

Type-specific HPV prevalence is reported in Fig 2a: HPV-16 was the most prevalent type which was detected in 4.74% of HIW and in 4.61% of SPW. No significant differences were observed in the patterns of distribution of the HIW and SPW groups, except for the identification of infections sustained by HPV-67 and HPV-34 in HIW, absent in SPW and infections by HPV- 42 and HPV-72 types in SPW, absent in HIW.

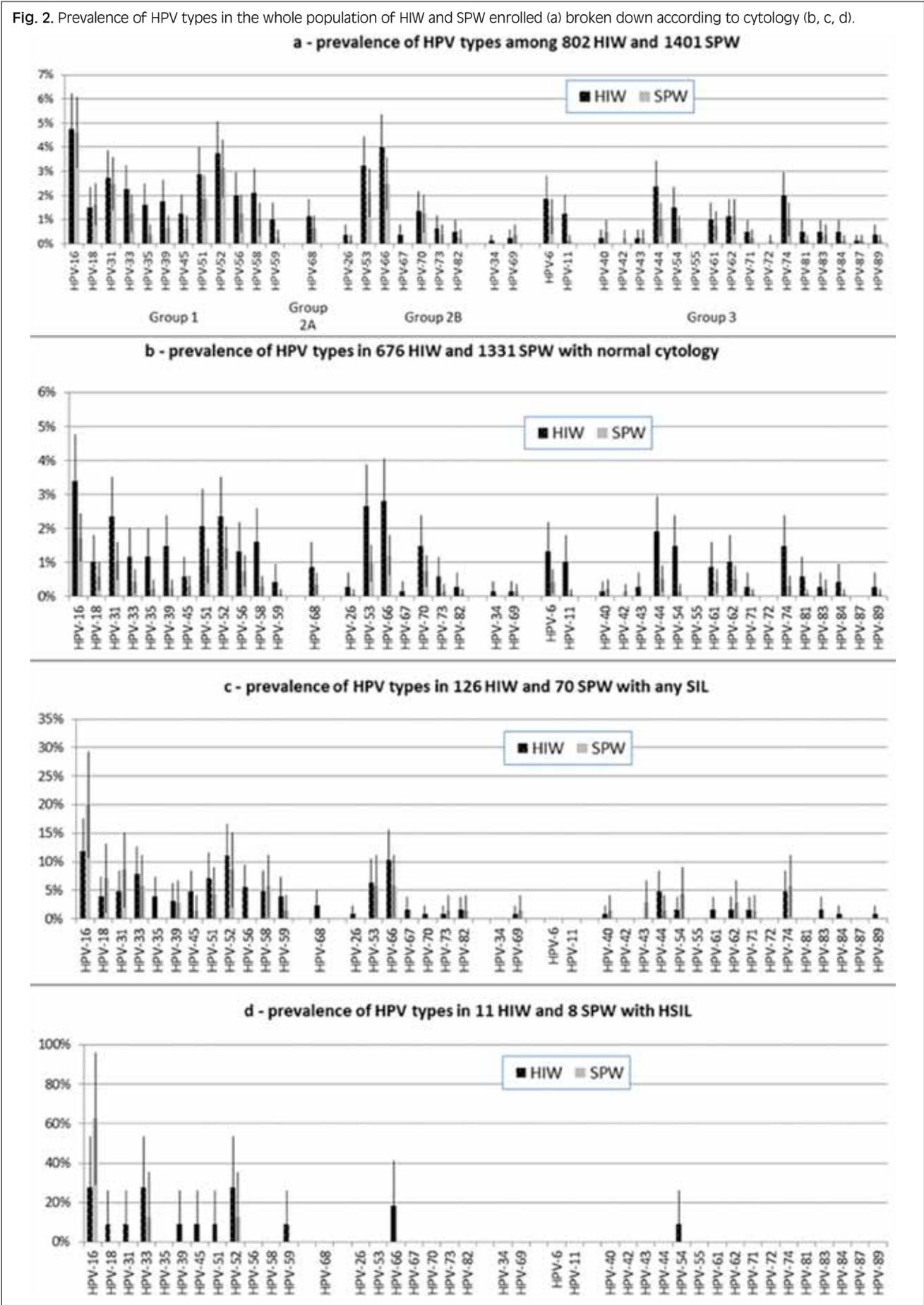
HPV typing stratified by cervical cytological results (Fig. 2b) showed very similar patterns in HIW and SPW with normal cytology. There is a lack of data concerning women with abnormal cytological results therefore we were unable to establish a different distribution pattern; however, HPV-16 is by far the most common type of HPV in women with abnormal cytology (any grade SIL, Fig. 2c) in SPW, while HPV-16 prevalence is similar to other high-risk HPV types (HPV-52, HPV-33 and HPV-66) in HIW. Several other HR-HPV types have been reported in HIW patients with cytological abnormalities (Fig 2c-2d). HSIL lesions sustained by non-HPV-16/18 types were 16.7% (1 out of 6) in SPW and 70% (7 out of 10) in HIW.

The cumulative prevalence of the two main oncogenic types (HPV-16/18), broken down by cytological outcome, is lower in LSIL and HSIL among the HIW than in the SPW and the general Italian population (Fig. 3).

Tab. II. Prevalence and OR with 95%CI of HPV-DNA by cytology in the HIW and SPW.

Cytology	HIW	%	SPW	%	P value	OR	95%CI
Normal	145	21.45	127	9.54	REF	1	
ASCUS vs normal	19	42.22	6	25.0	0.0351	2.774	1.074 to 7.161
LSIL vs normal	54	77.14	26	68.42	0.0290	1.819	1.076 to 3.076
HSIL vs normal	10	90.91	6	75.0	0.6082	1.460	0.5159 to 4.130

Fig. 2. Prevalence of HPV types in the whole population of HIW and SPW enrolled (a) broken down according to cytology (b, c, d).



Tab. III. Comparison of multi-type HPV infections according to cytology in HIW and SPW.

		Multi-type HPV/ HPV+ve	Prevalence	95%CI		P value	OR (95%CI)
Normal cytology	HIW	65/119	54.62%	45.68%	63.57%	0.0112	2.016 (1.184 to 3.435) ref
	SPW	40/107	37.38%	28.22%	46.55%		
Any SIL	HIW	40/75	53.33%	42.04%	64.62%	0.3117	1.600 (0.7166 to 3.572) ref
	SPW	15/36	41.67%	25.56%	57.77%		
HSIL	HIW	4/10	40.00%	9.64%	70.36%	0.5879	3.333 (0.2756 to 40.31) ref
	SPW	1/6	16.67%	13.15%	46.49%		

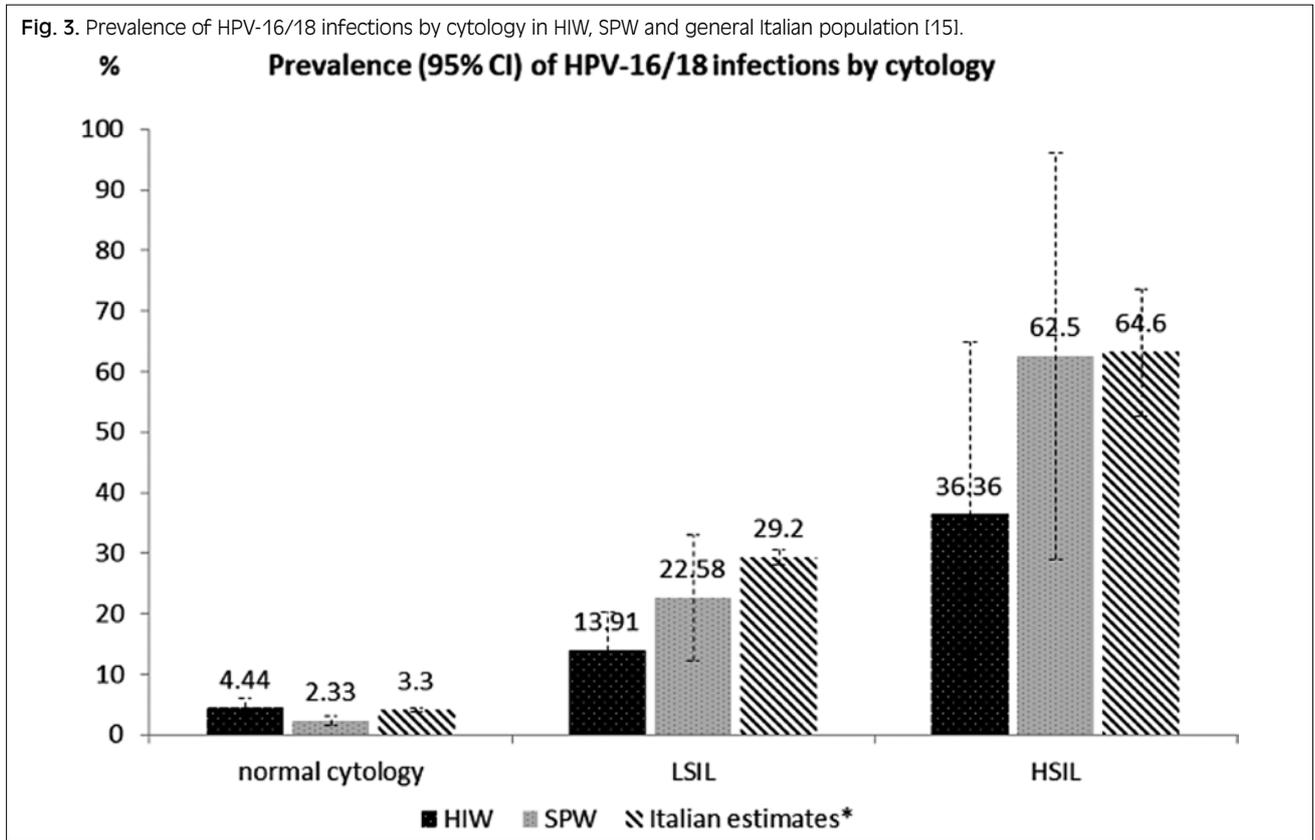
Multi-type HPV infections (2 to 9 HPV types) occurred in 54.62% (95%CI 4.68-63.57) and 37.38% (95%CI 28.22-46.55) of HIW and SPW with normal cytology respectively (p 0.011). No differences were observed in the prevalence of multi-type HPV infections in women with cytological lesions (any grade or HSIL) (Table III).

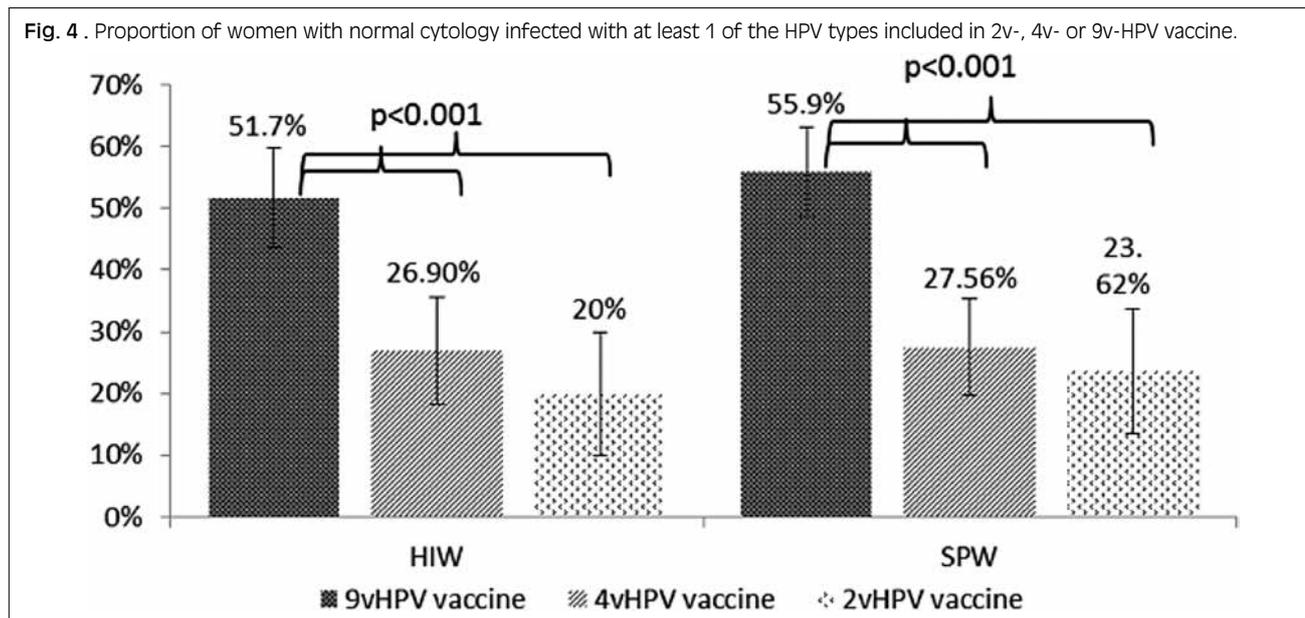
**INFECTION FROM VACCINE HPV-TYPES IN WOMEN WITH NORMAL CYTOLOGY**

No differences were observed in the proportion of infections sustained by at least one of the HPV types included in 2v-, 4v-, or 9v-HPV vaccines between HIW and SPW with normal cytology: 20.0% vs 23.62% for 2v-, 26.90% vs 27.56% for 4v-, and 51.7% vs 55.9% for 9v-HPV vaccine respectively. A highly significant difference in potential coverage was observed when we compared the 9v-HPV vaccine with the 2v- and 4v-HPV vaccines in both HIW and in SPW (Fig 4).

**Discussion and conclusions**

Worldwide, 69.4% of invasive cervical cancers are caused by HPV-16/18 infections [15]. In Italy, current estimates indicate that approximately 3.3% of females with normal cytology were infected with these two genotypes and the prevalence increased with disease progression [15]. The data reported in our study closely resemble the figures for SPW, even a slightly lower prevalence of HPV infection was observed in women with normal cytology; a comparable distribution of HPV types in women with normal cytology was found for HIW as well as a relatively lower presence of HPV-16/18 infections in the HSIL lesions compared to the Italian figures. HPV type distribution stratified by cytological results showed a substantial equivalence between women with HIV and SPW in normal cytology: HPV-16 is the most common viral type in both populations, followed by HPV-52, 66, 31, 53 for SPW and by HPV-66, 53, 52, 31 for HIW. Due to the





limited number of cases with abnormal cytology observed in this study, it was impossible to identify statistically significant differences of distribution between HIW and SPW. However, although HPV-16 is by far the most common type expressed in HSIL of SPW, there is a greater heterogeneity of genotypes in women with HIV infection and HSIL. Keller et al. [16] highlighted that HIV-infected women with HPV-16 infection and normal Pap test results have a similar pre-cancer risk as those with LSIL and therefore referral for colposcopy is warranted. However the presence of non HPV-16/18 infections was observed in 70% and 16.7% of HSIL in HIW and SPW respectively. McKenzie et al. [17] reported that cervical dysplasia specimens from 23 HIV infected women were infected (55%) by non-16/18 high-risk HPV types. If confirmed by larger cohorts, this finding may have major implications in the screening and triage strategies for women infected with HIV. As described by several Authors [18-19], it was observed that HIV-infected women have a higher prevalence of HPV and multi-type infections than the control group. A high prevalence of rare HPV types was detected in HIW, both in normal cytology and in cervical abnormalities and to date little is known about their carcinogenicity. The IARC classification and the analysis of rare HPV types show a wide variability in the carcinogenic potential of the rare and common HPV types found in normal cytology [1, 2]. In HIV-infected women, variability towards oncogenesis can be further influenced by the simultaneous presence of other cofactors, in particular cell-mediated immunosuppression [5], while the additional risks associated with each HPV type in multi-type infections is difficult to establish. Although no statistically significant differences were found due to lack of data, the description of viral types in the cervical specimens of HIV-infected women and correlation with the severity of cytological lesions can contribute to the mapping of HPV-related pre-invasive or invasive disease and provide a better risk stratification of disease evolution

in HIV-infected women. Even in the context of primary prevention through vaccination, the wide distribution of viral types found in high-grade lesions of HIV-infected women demonstrates the need for multivalent vaccines. This means that a primary prophylaxis with a 9v-HPV vaccine could have prevented infections in over 50% of the women included in this study, whether HIV infected or not. The assessment of the impact of HPV vaccines in the study population shows how immunization with a 9v-HPV-vaccine could prevent a significantly higher proportion of viral infections, both in HIV-infected people and in the control population, than 2v- and 4v-HPV vaccines. The ICC preventable fraction in the general population increased by 12-19% when the 9v-HPV vaccine was introduced due to the addition of 7 high risk HPVs to the 2v or 4vHPV vaccines [10, 20, 21] which could be even higher in HIV-infected people considering the wide range of HPV types observed in progressive diseases.

## Acknowledgements

This study was funded by Health General Direction, Regione Lombardia, Italy (DGR 10813/2009).

The sponsor was involved in the study design, but not in collection, analysis, interpretation of data, or writing of the paper. All authors had full access to all the data in the study. All the authors declare that they have no conflict of interest with the sponsor.

## Authors' contributions

GO: conceived and coordinate the study, evaluated the results and wrote the manuscript. ET: coordinated and contributed to the laboratory testing for HPV-DNA and HPV genotyping; contributed substantially to the manuscript writing. MMF, AM: contributed to the recruitment of the

participants, the acquisition of the clinical and epidemiological data, and critically revised the manuscript. SB, ERF, AA: contributed to the HPV detection and typing of the cervical samples and contributed to the manuscript writing. FM: contributed to the acquisition of the clinical and epidemiological data, to the samples handling and storage, and critically revised the manuscript. NZ: contributed to the HPV typing of the cervical samples and critically revised the manuscript. GR: contributed substantially to the conception, design and supervision of the study, and critically revised the manuscript.

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■ Received on July 7, 2017. Accepted on October 19, 2017.

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