Testing for human papillomavirus and measurement of viral load of HPV 16 and 18 in self-collected vaginal swabs of women who do not undergo cervical cytological screening in Southern France

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Testing for human papillomavirus and measurement of viral load of HPV 16 and 18 in self-collected vaginal swabs of women who do not undergo cervical cytological screening in Southern France

Running title: HPV testing of self-collected samples in France

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Abstract

Self-sampling using vaginal swabs could be a valuable alternative to screen for cervical cancer for women who do not attend regular cytological screening. The aim of this study was to determine the prevalence of high and low-risk HPV types and of HPV type 16 and 18 DNA load in self-collected vaginal swabs from 35- to 69-year-old Southern French women of low socioeconomic level or migrant populations who do not attend regular cervical screening. A good concordance (93.1%) was found between cervical brush and vaginal swabs in 29 samples. Self-collected vaginal swabs were examined from 120 women. HPV infection was found in 28 women (23.3%; median age 48 years), 17 (14.1%) of whom harboured high-risk HPV types. HPV type 16 was the high risk type found most frequently, followed by types 53, 31, 18, 58, and 66. The low-risk type detected most frequently was HPV type 6, followed by types 61, 70, and 81. The mean HPV 16 and 18 load was $6.3 \log_{10}$ copies/ 10^6 cells and $2.4 \log_{10}$ copies/ 10^6 cells, respectively. These results suggest that vaginal self-swabs can be a reliable tool for cervical cancer screening in non-attending and inadequately screened elderly women.

Key Words: human papillomavirus; viral load; cervical cancer; self-obtained vaginal swabs; low economic class women; high-risk and low-risk HPV types.

INTRODUCTION

Cervical cancer is the second leading cause of cancer deaths among women worldwide, with an estimated incidence of 500,000 cases each year and a quarter of a million deaths [Franco et al., 2003]. In France, there are more than 3,600 new cases of cervical cancer each year, resulting in more than 1000 deaths per year. Human papillomavirus (HPV) is the major cause of cervical cancer in women. Cytology-based screening (“pap-smear”) is the major method of screening for cervical cancer. However, the sensitivity of this method is limited, and the efficacy of cytology-based screening programs largely depends on the coverage of the female population. Thus, approximately 60% of cervical cancers are not adequately screened [Sawaya et al., 1999]. In France, cervical cancer screening programs are unorganized, and approximately 40% of women do not participate in such programs. As such, French women are at a major risk of being diagnosed with cervical cancer [Schaffer P et al., 2000].

Recent studies have demonstrated that performing HPV DNA testing, in addition to cytology, is useful for primary cervical cancer screening [Mayrand et al., 2007; Naucler et al., 2007; Bulkmand et al., 2007]. Detection of HPV type 16 or 18 in cervicovaginal samples correlates with a higher risk for dysplasia or cervical cancer, even if cervical cytology is normal [Josefsson et al., 2000; Wright et al., 2000; Khan et al., 2005; Brinck et al., 2006]. Moreover, the incidence of severe dysplasia and cervical cancer within 10 years is elevated by HPV type 16/18 [Khan et al., 2005], and a relationship between HPV 16 viral load and the severity of cervical lesions has been previously demonstrated [Carcopino et al., 2006; Gravitt et al., 2007; Saunier et al., 2008].

Cultural barriers, reluctance towards vaginal speculum examination, and refusal to undergo routine pelvic examinations in the absence of symptoms are the most common reasons for which women fail to undergo regular cervical screenings [Piana et al., 2007]. Several studies have shown that detection of HPV by vaginal self-sampling can be a valuable alternative for women refusing cytological screening and can also help to improve coverage [Cuzick et al., 1995; Holanda et al., 2006; Karwalajtys et al., 2006; Brinck et al., 2006; Stenvall et al., 2007; Bais et al., 2007]. Vaginal self-sampling is as sensitive as physician-collected endocervical cytobrush sampling for detecting high-risk-HPV (HR-HPV) DNA [Hillemanns et al., 1999; Sellors

et al., 2000; Gravitt et al., 2001; Nobbenhuis MA et al., 2002; Petignat et al., 2005; Brinck et al., 2006; Karwalajtys et al., 2006; Petignat et al., 2007). To date, few studies have demonstrated the reliability of self-sampling devices for the evaluation of oncogenic HPV viral load [Daponte et al., 2008].

The aim of this feasibility study was to assess the prevalence of HR- and low-risk (LR)-HPV infection, as well as of HPV 16 and 18 DNA load, in women aged 35 years and older, from low socioeconomic backgrounds and/or migrant populations in Marseille, France who do not attend regular cytological screenings for cervical cancer (referred to hereafter as “non-attending”). For this purpose, self-collected vaginal swabs were evaluated as a valuable tool for virological investigation.

Samples and Methods

Preliminary assessment of vaginal swabs

Physician-collected vaginal swabs and auto-sampled cervical brushes were compared for HPV diagnosis and HPV 16 and 18 quantitation. A total of 29 paired samples from 29 women referred to the gynecological outpatient department for cytological screening or for colposcopy were obtained. The paired samples were processed in the same assay. The median age of the women under study was 38 years (IQR: 30-48).

Collection of cervical and vaginal swabs, HPV DNA quantification, and HPV genotyping

Flocked swabs (Copan Diagnostics) were used as self-sampling devices, and cervical brushes were used by physicians. To compare self-collected vs. physician-collected samples, all vaginal samples were suspended in Universal Transport Medium (UTM) tubes, sent to the Virology Laboratory of the Marseille University Hospital, and stored at -20 °C until use.

Study population: a pilot sampling campaign was organized in 2 suburbs in the northern part of Marseille, where the rate of low socioeconomic level is high (37,2 to 45,5%). The women of low socio-economic level and/or from migrant populations selected for the study were aged 35 to 69 years and without a pap smear indexed in the National Insurance Register in the past 2 to 3 years. By individual mailing, the women were randomly either re-invited for a pap smear or invited to perform a self-sampling of their vaginal fluid in a medical analysis laboratory close to their home..

UTM tubes were stored at -20°C at the local medical analysis laboratory and transmitted in dry ice to the Virology Laboratory of the University hospital.

A total of 5,360 invitation letters were sent and 123 vaginal samples were received (2.29%). Out of these 123 persons, women infected by HPV were re-invited for a pap-smear test and, if necessary, a colposcopy.

Virological Methods

DNA extraction:

After thawing, 250 µl of vaginal cell suspension was used for DNA purification using QIAamp DNA Mini Kit (QIAGEN, Courtaboeuf, France) modified as follows: samples were incubated at 56°C for 2 hours in lysis solution containing proteinase K. The DNA was eluted in 100 µl of elution buffer and stored at -20°C until use.

HPV L1 gene amplification by single or nested PCR:

For primary PCR amplification, 5µl of vaginal cell DNA, 1µl of 10µmolar degenerate MY09:5'GCMCAGGGWCATAATAATGG3'/MY11:5'CGTCCMARRGGAWACTGATC 3' primers, 0.2 µl of Hotstar polymerase (Qiagen), 5X ready to use polymerase buffer, Dnase and Rnase free water were added in a final volume of 50µl. The PCR was performed at 95°C 15 min followed by 40 cycles (95°C 30s, 52°C 45s, 72°C 1min). A second amplification was performed using the same procedure, and 1µl of the first amplification was used as sample. The primary and second amplification products were analysed on BET agarose gel.

HPV type determination by sequencing, cloning, and phylogenetic analysis:

450bp PCR amplicons were purified and sequenced on ABI PRISM 3110 (Applied Biosystems) using Big Dye terminator V1.1 Cycle sequencing Kit (Applied Biosystems). The sequence obtained was introduced in a phylogenetic tree that contained sequences of HPV L1 gene available in NCBI nucleotide database <http://www.ncbi.nlm.nih.gov/nucleotide/>. Ambiguous determination was confirmed in silico by NCBI Blast analysis <http://blast.ncbi.nlm.nih.gov/Blast>.

Cloning of PCR Product: when the sequence profile revealed a mixture, the PCR product was cloned using pGEM-T easy System (Promega, Madison, WI); about 20 clones were sequenced and HPV types were determined by phylogenetic analysis.

HPV types considered as high-risk or probably high-risk included HPV 16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82; while HPV types with low-

risk or unknown oncogenic risk included HPV 6, 11, 34, 40, 42, 44, 54, 61, 62, 70, 71, 72, 81, 83, 84, and 89, based on the classification by Munoz et al [2006].

HPV 16 and 18 screening and viral load determination:

To quantify the HPV 16 and 18 DNA, a quantitative, duplex real-time (RT)-PCR was performed as previously described [Carcopino et al., 2006]. The quantitation plasmid used contained the three target sequences of interest: HPV 16 (on E6 gene), HPV 18 (on E7 gene), and human albumin gene (on exon12). The plasmidic solution made it possible to obtain a quantification scale from 10^7 to 1 copy for 5 μ l of solution. Positive, negative, and no template controls were introduced in each reaction plate. In the experimental conditions used, the cycle threshold (CT) values for the standard curve of the RT PCR varied from 17 CT (10^7 copies) to 39 CT (1 copy) and the slope varied from -3.0 to -3.2. This allowed a lower detection limit of HPV16 or HPV 18 at 5 copies per 5 μ l sample. It was established that the samples with a human albumin CT value higher than or equal to 31 (10^2 copies of albumin gene, or 50 human nuclear cells) were excluded from the analysis in order to avoid false negative results. This method allowed HPV 16 and 18 and albumin gene copy number to be quantified in the same assay. So, HPV viral load could be expressed as HPV copies per cell or per million cells. All the specimens were tested by both MY09/MY11 nested PCR and RT-PCR HPV16/18/human albumin.

Statistical analysis

Data were entered into an EXCEL spread sheet and analysed using EpiInfo software version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA).

The Yates-corrected chi-square test or the Fisher exact test were used to compare proportions. Continuous variables were compared by using either the ANOVA test or the Mann-Whitney/Wilcoxon test according to the results of the Bartlett test for inequality of population variances. For all analyses, a *P*-value of <0.05 was

considered statistically significant. For type-specific HPV prevalence, all genotypes from single and multiple infections were computed individually. Agreement between tests was measured by Cohen’s kappa statistics.

RESULTS

Preliminary assessment of vaginal swabs

Results from vaginal swab versus endocervical brush samples are shown in Table I. Of the 29 participants tested, 12 (41.3%) had CIN1 or CIN2/3 lesions whereas HPV infection was detected in 16 (55%) swab and 17 (58.6%) endocervical brush samples, respectively, by either genotyping or RT-PCR. HPV 16 and 18 DNA loads were comparable in both vaginal swabs and endocervical brush samples (mean HPV 16 viral load: 6.5 vs. 6.2 log, p = 0.17; Table I). For 27 women, HPV types were concordant (12 were concordant positive and 15 negative) in both vaginal swabs and endocervical brush samples (Table II). The overall concordance level between vaginal swabs and endocervical brush samples was 93.1% (k= 0.86). There were 2 cases of discordance between vaginal swabs and endocervical brush samples: one case of cervical brush sample harbouring multiple HPV types (6, 53, and 58) while vaginal swabs harboured HPV type 6, and one case of vaginal swabs harbouring HPV type 61 while the cervical brush sample was negative (Table I; Table III). The frequencies of HR- and LR-HPV types are presented in Table III. Infection with multiple HPV types was detected in 3 of 16 HPV DNA positive vaginal swabs (HPV 6+16, HPV 16+31, HPV 16+58) and in 4 of 17 HPV DNA positive endocervical samples (HPV 6+16, HPV 16+31, HPV 16+58, HPV 6+53+58).

Assessment of the prevalence of HR- and LR-HPV infection and of HPV 16 and 18 DNA load in self-collected vaginal swabs

Data from 123 recruited women were recorded. To avoid false negative results, the samples of 3 women (40-, 49-, and 50-years-old) were excluded from the study because high CT values, that is, ≥31 (10² copies of the albumin gene in 50 human nuclear cells), revealed a low cellular content of the sample. Samples from 120 (97.6%) women were further analysed (median age: 48.2±9 years; interquartile range

(IQR): 42-54; range: 35-69). Forty-one (34.2%) women were ≥ 50 -years-old and 18 were ≥ 60 -years-old. Of the 120 women sampled, 28 (23.3%) were infected with HPV, and 17 (14.1%) of them harboured HR-HPV types. HR-HPV types most frequently found were HPV-16 (7), 53 (5), 31 (4), 18 (2), 58 (1), and 66 (1) (Table IV).

LR-HPV types most frequently found were HPV type 6 (6), 61 (5), 70 (2), and 81 (2). Six (21.4%) women were infected with multiple HPV types (≥ 2) and two of them were infected with HR-HPV types (≥ 1). HPV types 83 and 84 of undetermined risk were found in 3 women. The median age of HR- and LR-HPV-infected women was 48-years-old (range: 37-61; IQR: 39-54 years) and 46-years-old (range: 37-59, IQR: 42-54 years), respectively. No differences related to age were found between HR- and LR-HPV-infected women ($p=0.6$). Among the 17 HR-HPV-infected women, 7 (41.1%) were ≥ 50 -years-old, while 4 (36.3%) of the 11 LR-HPV-infected women were ≥ 50 -years-old ($p=1.0$). As shown in Table V, no age-related differences were found between groups of HPV-uninfected, HR-HPV infected, LR-HPV-infected, and multiple HPV-infected women ($p=0.6$). The mean HPV 16 load, quantified from 5 women, was $6.3 \log_{10}$ copies/ 10^6 cells; the mean HPV 18 load, quantified from 2 women, was $2.4 \log_{10}$ copies/ 10^6 cells (Table IV). HPV 16 DNA loads did not differ significantly with age ($p=0.78$). No correlation was found between the women's age and their oncogenic viral load by linear regression ($r^2=0$, $p=0.70$).

After discovery of the 28 positive DNA tests, 15 women among those harbouring HR-HPV types were referred to their physician. Eight women were referred for cytology (4 HPV 16, 1 HPV 18, 3 other HPV types): 1 was abnormal, 5 normal, 2 without information. Among the 17 women harbouring HR-HPV types, six were referred to colposcopy (1 HPV type 18 and 5 other HR-HPV types): two women had high-grade lesions and one had surgery.

DISCUSSION

The preliminary results obtained from the comparison between vaginal swabs and endocervical brush samples were highly concordant with respect to HPV type diagnosis and HPV 16 and 18 load determination. These results validated the use of vaginal swabs for virological investigation. Therefore, in the second part of the study 123 women were enrolled who performed vaginal self-sampling for HPV analysis. In

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this population, the prevalence of HPV infection was relatively elevated (23.3%) in women whose median age was 48.2 years. Moreover, 14.1% of the women were infected with HR-HPV types and of them, 21.4% were infected with multiple HPV types. These results contrast with data from previous studies conducted in Canada and in the Netherlands, showing that 6% of women aged 30 to 69 years [Mayrand et al., 2007] and 4.5% of women aged 29 to 56 (median age: 41.0 years) [Bulkmans et al., 2007], respectively, exhibited HPV DNA positivity. Usually, the prevalence of HPV infection decreases with age [Melkert et al., 1993; Munoz et al., 1996; Kitchener et al., 2006] with the highest prevalence rate observed before the age of 30. Before the age of 30, the HPV test has a poor positive predictive value since most HPV-infected women will clear the infection within 7 to 10 months [Ho et al., 1998] and will not develop lesions. In one recent Italian study, the prevalence of HR-HPV genotypes was 13.1% in patients aged 25 to 34 years and 5.8% for women aged 35 to 60 years [Ronco et al., 2008]. The present study shows a high prevalence rate of HPV infection in women aged 35 to 69 years. These results agree with several studies that have reported a second peak of HPV prevalence in women 50 years and older [Herrero et al., 1997; Sellors et al., 2002; Baay et al., 2004] and a particularly high prevalence of HPV infection (15.5%) in women 60 years and older [Levert et al., 2000]. In addition, an Italian study has also reported an increased prevalence of HPV in women aged 64 years and older (12.2%) [Agarossi et al., 2009]. In the present study, the reasons for the high prevalence of HPV infection in older women are probably multifactorial and include the low socioeconomic level of women who are reluctant to undergo gynecological examination, reactivation of latent infection in the postmenopausal period due to decreasing specific immunity, and/or acquisition of new infections in sexually active older women.

The HR-HPV type most frequently detected in this population was HPV 16, followed by HPV, 53, 31, 18, 58, and 66. The observed frequencies of these HPV types are in line with a recent Italian study of 9,947 women self-referred for cervical cancer screening [Agarossi et al., 2009]. In addition, a meta-analysis of HPV positive women with normal cytology reported HPV 16, 18, 31, 58, and 52 as the most common types worldwide [De San Jose et al., 2007]. A more recent study [Lindau et al., 2008] analysing self-collected vaginal samples from 1,550 women in Chicago showed a high prevalence of HPV types 61, 31, 52, 58, and 66 in women aged 57 to 85. One could expect a potential protective effect of the vaccines Gardasil and Cervarix on

the majority of HR-strains circulating in France since both vaccines confer a certain degree of cross-protection against HPV 31, 33, 45, 52, and 58 [Harper et al., 2006; Paavonen et al., 2008]. Nevertheless, the present study underscores the need to regularly survey the worldwide distribution of HPV in target populations in order to adapt vaccines for protection against the prevailing HPV types.

The LR-type most frequently detected was HPV 6 followed by HPV 61, 70, and 81, which is in line with a recent French epidemiological study [Aubin et al., 2008].

In the present study, infection with multiple HPV types was found in 21.4% of HPV-infected women, a relatively low rate given that direct sequence analysis detects fewer multiple HPV types than methods such as reverse hybridization methods, Linear Array, or DNA chips. The prevalence rate of infection with multiple HPV types reported in the literature ranges from 20% to 50% of infected subjects [Nielsen et al., 2008]. Although it is not definitively known whether co-infection with multiple HR-HPV types increases a woman's risk for cervical neoplasia [Bosch et al., 2002; Van der Graaf et al., 2002], a study by Agarossi et al. [2007] showed that patients harbouring multiple HR-HPV types had a 30% higher risk of presenting abnormal cytological results.

One of the major observations of this study, with a potential impact in terms of public health, was that an HPV 16 load $>5.2 \log/10^6$ cells was found in 3 patients, which is very close to the cut-off value of $5.3 \log/10^6$ cells predictive of the persistence of HPV infection and its progression towards the appearance of precancerous lesions as shown by Monnier-Benoit et al. [2006].

This work provides important information on the unexpected high rate of HPV and HR-HPV infection in 35 to 69-year-old migrant non-attending women of low socioeconomic levels in France. The results suggest that this category of women is probably at increasing risk for development of cervical cancer. Moreover, HPV type 16, which is associated with a high risk for the appearance of cervical lesions [Herrero et al., 1997; Ho et al., 1998; Woodman et al., 2001], was detected in 2 women aged between 45 and 50 years and 2 women aged 50 years and older. Altogether, these data have to be put back in their context; indeed, in France, screening is organized in only 5 areas and stopped at the age of 65 years. Elsewhere in France, screening generally decreases after menopause. Meanwhile, a tendency towards increased cervical cancer incidence and mortality is observed from the age of 65 (incidence of 17 cases p100.000 persons/year; rate of mortality between 5.3

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2 and 11.6 p100000 persons/year in women aged 65 to 80 years [Remontet L et al.,
3 2003].
4 One of the limitations of the present campaign was the low-response rate to self-
5 sampling and follow-up cytology which was related mainly to low socio-economic
6 status and illiteracy; in the next screening campaign, it will be important to determine
7 the actual proportion of women with a persistent infection who have histological CIN-
8 2-3 lesions. To this end, a heading requiring the women's telephone number and
9 physician's last name and address has been added to the record form accompanying
10 the self-sampling device. Thus, HR-HPV-infected women will be easily joined by
11 phone and strongly encouraged to follow-up cytology and colposcopy. Moreover,
12 after one year, HR-HPV-infected women will be re-invited to perform again a self-
13 sampling. In case of persistent HR-HPV infection, they will receive an information
14 letter inviting them for a suitable follow-up and the non-responders will be joined by a
15 social worker. Another possible limitation of this study was the lack of individual data
16 regarding the socio-economic status of the women contacted, which makes it difficult
17 to determine if the prevalence estimates can be applied to the targeted population.
18 Since collecting individual data is not allowed in France by the National Commission
19 for Information Technology and Civil Liberties, the targeted population was selected
20 in 2 districts of the city with a high deprivation index calculated from census data
21 recorded by the National Institute for Statistics and Economic Studies and from the
22 National Health Insurance.
23 Overall, the results reported herein underline the importance of collecting follow-up
24 data that combine cytology/histology and HPV detection in elderly women, especially
25 since the clearance rate of HPV infection in these women is unknown. Self-sampling
26 could be used as a screening tool for elderly women who are not adequately
27 screened. Moreover, pap-smear is often uninterpretable in menopausal women.
28 Individual screening tests are performed less regularly after menopause and are
29 officially stopped after the age of 65. In this pilot feasibility study, it was demonstrated
30 that self-sampling provides only 2.4% (3 of 123) of uninterpretable samples related to
31 the paucicellularity of the samples.
32 In conclusion, this study, using self-collected vaginal samples for cervical cancer
33 screening in a target population of 35- to 69-year-old women, of low socio-economic
34 status who do not attend regular cytological screenings, demonstrates that there is a
35 high prevalence of HR-HPV infection, with an elevated HPV 16 viral load in some

cases, and a broad diversity of HPV genotypes. A second broader screening study is currently underway in the Marseille area. In addition, this work is relevant in view of developing a safe and effective vaccine against the most frequent HR- and LR-HPV types.

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Table I. HPV test results for 29 paired vaginal samples and cervical brush samples

	Vaginal sample results	Endocervical brush sampling method results
HPV infection, n (%)	16 (55)	17 (58.6)
Positive genotyping results*, n (%)	13** (81.3)	13*** (76.5)
Positive Real-time PCR HPV 16 or 18	6	6
Mean HPV 16 load log ₁₀ copies/10 ⁶ cells (n =4)	6.5	6.2
Mean HPV 18 load log ₁₀ copies/10 ⁶ cells (n=2)	2.7	3.9

- *Including ≥1 HPV type
- ** One vaginal sample harboured HPV type 6 only while endocervical sampling harboured HPV types 6+53+58
- *** One sample was PCR negative by endocervical brush and positive by vaginal sampling (HPV type 61)

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Table II. Concordance of genotyping results between vaginal swabs and endocervical brush samples

Vaginal swabs	Endocervical brush samples		
	Positive	Negative	Total
Positive	12	1	13
Negative	1	15	16
Total	13	16	29

Concordance rate: 93.10% (k = 0.86) (p<0.05)

Table III. Distribution of high-risk and low-risk HPV types from 29 paired vaginal swabs and endocervical brush samples by RT PCR and/or genotyping

		Vaginal Swabs	Endocervical brush samples
	HPV type	N (%)	N (%)
High-Risk	16*	4 (25)	4 (23)
	18**	2 (12.5)	2 (11.8)
	31	3 (18.7)	3 (17.6)
	33	2 (12.5)	2 (11.8)
	52	1 (6.2)	1 (5.8)
	53***	0	1 (5.8)
	58	3 (18.7)	4 (23.5)
Low-Risk	6	2 (12.5)	2 (11.8)
	61	2 (12.2)	1 (5.8)
	81	1 (6.2)	1 (5.8)
Multiple HPV infection	6+16	1	1
	16+31	1	1
	16+58	1	1
	6+53+58	0	1

- *Positive by Real-Time PCR and/or genotyping and including those detected in multiple HPV infection
- ** Positive by Real-Time PCR only
- *** Included in multiple HPV infection

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Table IV. Main virological characteristics of 28 HPV-infected females

HPV infection, n (%)		28 (23.3%)
Number of samples with high-risk HPV types		17(14.1%)
Number of samples with ≥ 2 HPV types		6 (21.4%)
Number of samples with ≥ 1 high-risk HPV type		2 (7.1%)
High-risk HPV types	16	7 (25%)
	18	2 (7.1%)
	31	4 (14.3%)
	53	5 (17.8%)
	58	1 (3.5%)
Low-risk HPV types	66	1 (3.5%)
	6	6 (21.4%)
	61	5 (17.8%)
	70	2 (7.1%)
	81	2 (7.1%)
Undetermined risk HPV types	83	2 (7.1%)
	84	1 (3.5%)
Mean HPV 16 load (n=5)		6.3
HPV 18 load (n=2)		2.4

Table V. Age of the different groups of women according to the HPV status

	Mean Age $\pm$ SD	Range	Median Age (IQR)
HPV uninfected women	48.5 $\pm$ 9.1	35-69	46 (42-54)
HR-HPV infected women	46 $\pm$ 8.5	37-59	42 (39-59)
LR-HPV infected women	48 $\pm$ 8.6	35-63	48 (39-63)
Multiple HPV infected women	55 $\pm$ 8.4	49-61	55 (49-61)

No significant difference according to age between the different groups of women, $p=0.6$ (ANOVA test)