Isolated Non-16/18 High-Risk Human Papillomavirus Cervical Infection: Re-Evaluation After One Year

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ABSTRACT

Introduction: High-risk human papillomavirus cervical infection is currently a well-established cause of cervical cancer. However, only a few women with persistent infections will develop cervical precancerous and malignant lesions. Approximately 20% of all cervical cancers are attributable to non-16/18 serotypes. This study aims to evaluate the results of our clinical approach to women with this infection.

Material and Methods: We conducted an observational and prospective study from September 2012 to September 2017, which included women with isolated non-16/18 high-risk human papillomavirus infection (with normal cytology). After re-evaluation, two groups were compared: women with spontaneous regression of the infection and women with persistent infection. Clinical and demographic data were analysed as well as the rate of progression to precancerous and malignant lesions.

Results: We included 165 women, of which 121 were re-evaluated with co-test at least one year later. After re-evaluation, 13.2% of women revealed precancerous lesions but only two (1.7%) of them presented high-grade squamous intraepithelial lesions. Sixty-seven women (55.4%) showed spontaneous regression of the infection and 54 women (44.6%) maintained it. Women with persistent infection developed more precancerous lesions (27.8%; p < 0.001) and high-grade squamous intraepithelial lesions (3.7%; p < 0.001). There was also an association between persistent infection and postmenopausal status.

Discussion: Human papillomavirus 16/18 cervical infection is associated with higher risk of cervical cancer when compared with other serotypes.

Conclusion: Re-evaluation with co-test one year after the diagnosis of isolated non-16/18 human papillomavirus infection seems to be a reasonable approach.

Keywords: Genetic Variation; Papillomaviridae; Papillomavirus Infections; Precancerous Conditions; Uterine Cervical Neoplasms

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted viral infection. Virtually all sexually active people will be infected during their lifetime and some may even be repeatedly infected.1,2 More than 100 types of HPV have been identified, but only 15 are considered oncogenic and have cervical tropism.3 These are classified as high-risk HPV (hrHPV) and cause nearly all cases of cervical cancer, the fourth leading cause of female cancer worldwide.4,6 However, not all high-risk types have the same carcinogenic potential, with non-16/18 hrHPV only being responsible for 20% of all cervical cancers.5,7

The majority of HPV cervical infections do not cause symptoms or disease, and resolve spontaneously within...
two years. Persistent infection may lead to precancerous lesions (low and high-grade squamous intraepithelial lesions) and evolve to cancer. Risk factors for HPV persistence are not well defined, but some have been identified: HPV type, viral load, duration of the infection, old age, HIV infection and immune suppression. Early first sexual intercourse, history of multiple sexual partners, parity and young age at first birth, co-infection with other sexually transmitted agents, tobacco use, and oral contraceptive use for more than five years have also been associated with HPV infection and cervical cancer.

Based on the fact that cervical cancer is a preventable disease, there is an attempt to screen all women at risk and to identify and treat precancerous lesions. There are currently three types of screening available: cytology, visual inspection with acetic acid and hrHPV testing. Despite being less specific, hrHPV testing is more sensitive, reliable and has a higher negative predictive value. Furthermore, incorporation of hrHPV testing into screening strategies has the potential to increase accuracy and the length of screening intervals. Thus, it was introduced as an adjunct to cytology screening (co-testing) and, in some facilities, as the primary screening test.

In Portugal, we have a comprehensive programme for cervical cancer prevention and control, which includes primary, secondary and tertiary prevention. However, it does not cover all the population and leaves room to local organization of opportunistic screening in many health centres and hospitals. According to previous studies, the prevalence of isolated HPV infection in Portuguese women ranges from 15.5% to 25.4%, and non-16/18 hrHPV are identified in higher proportions than serotypes 16 and 18. Cytological results were reported according to the new Bethesda system.

We conducted an observational prospective study from September 2012 to September 2017, which included women aged 30 to 65 with diagnosis of isolated non-16/18 hrHPV infection (designation meaning that no other hrHPV was identified and colposcopy evaluation was normal). All women were being followed at our department and the date of diagnosis corresponded to the date of inclusion in our convenience sample. The co-tests performed included liquid-based cytology (ThinPrep® Pap test) and hrHPV testing (Cobas® test, Roche). This test simultaneously provides pooled results on hrHPV (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and individual results on HPV 16 and 18. Cytological results were reported according to the Bethesda system.

Based on the result of an isolated non-16/18 hrHPV infection, we informed all women that re-evaluation with co-test should be performed one year later. We contacted every woman who did not attend the follow-up one year after the diagnosis. We also analysed clinical and demographic data including age, age of menarche and menopause, parity, age of sexual debut and number of sexual partners, tobacco, oral contraceptive use (rate of current users) and co-infection with HIV. After re-evaluation with co-test, we formed and compared two groups: women with spontaneous regression of the infection and women with persistent infection. We examined the rate of progression to precancerous and malignant lesions. We performed statistical analysis with t-test and \( \chi^2 \) (IBM® SPSS® Statistics 24) and we considered \( p \) values lower than 0.05 as statistically significant.

**RESULTS**

We included 165 women in this study. The demographic and clinical characteristics analysed are represented in Table 1. Twenty-two (13.3%) women had HIV infection, but most had negative viral load.

Among all women, only 121 women were re-evaluated with co-test: 86.8% (n = 105) after one year, 10.7% (n = 13) after two years, 1.7% (n = 2) after three years and 0.8% (n = 1) after four years. Thirty-four women (20.6%) did not return for further evaluation, while others (6.1%) will only be re-evaluated in the future.

After re-evaluation, 16 women (13.2%) revealed precancerous lesions: four (3.3%) atypical squamous cells of undetermined significance (ASCUS), ten (8.3%) LSIL and two (1.7%) HSIL. Only one of the women who developed LSIL showed spontaneous regression of the infection. HSIL were not confirmed by histological evaluation. We did not identify any malignant lesions.

Sixty-seven women (55.4%) had spontaneous resolution of the infection. Fifty women (41.3%) maintained isolated non-16/18 hrHPV infection, two (1.6%) got co-infected
(one with serotype 16 and the other with serotype 18) and two (1.6%) developed an isolated 16 or 18 infection. None of these four women developed precancerous lesions, but 27.8% of those infected with non-16/18 hrHPV revealed cytological abnormalities (3.7% HSIL).

There was an association between persistent infection and the development of precancerous lesions ($p < 0.001$) and HSIL ($p < 0.001$). Postmenopausal status was also associated with persistent infection ($p = 0.01$). Besides that, we did not find other statistical differences in the analysed demographic and clinical characteristics between women who showed spontaneous resolution of the infection and those who revealed persistent infection (Table 1).

**DISCUSSION**

Persistent hrHPV cervical infection is associated with cervical dysplasia and malignancy, which typically develops within five to ten years.\(^2,4,22,23\) Furthermore, the risk of progression with persistent non-16/18 hrHPV cervical infection is lower than persistent infection with serotypes 16/18, but it is still higher compared to no infection.\(^24-28\)

In our study, we re-evaluated 121 women (73.3% of our sample) who had an isolated non-16/18 hrHPV cervical infection. The majority (86.8%) of them were re-evaluated after one year. The rate of spontaneous regression of the infection (55.4%) and the rate of development of precancerous lesions (13.2%; 1.7% HSIL) were consistent with previous studies.\(^25,29,30\) Due to the lack of information regarding all the results of previous screenings performed in these women, we do not know the real duration of these infections. Additionally, we do not know how much time passed until the development of a lesion. Nevertheless, this represents a frequent scenario, since not all women are regularly screened and the risk of cancer is real. Furthermore, the group of women with persistent infection developed more precancerous lesions (27.8%) and HSIL (3.7%), which is in agreement with previous publications that identified the persistence of infection as the major risk factor in the development of cervical cancer.\(^7-9\)

The average age at re-evaluation was 46 years old and almost 31% of these women were postmenopausal. In our institution we follow a high number of postmenopausal women and women infected with HIV. Knowing that the risk of persistent hrHPV cervical infection in young women is lower than in older women,\(^28,30\) our population might require special care and follow-up. In fact, we found an association between postmenopausal status and persistent infection.

Regarding older women, previous studies suggest that the latency of HPV is possible, as it is in women with HIV infection.\(^31-33\) Besides that, HIV co-infection is also a known risk factor for the development of cervical cancer.

Even though we have performed an analysis of previously identified risk factors for persistence of the infection, we did not find other statistical differences in the analysed demographic and the clinical characteristics between women who had spontaneous resolution of the infection and those with persistent infection.

The relatively short duration of follow-up and small number of cases included in our sample may represent a limitation of this study, but until now no similar studies were performed in Portugal. Our results might not be generalizable to all populations because baseline characteristics might be different. Still, since HPV testing is not available in all health care centres in our country, recognising its value, especially in groups at higher risk of developing cervical lesions, seems to be essential. According to our results, to the demographic and clinical characteristics of our population and even though non-16/18 hrHPV are responsible for a minority of all cases of cervical cancer, our approach and results seem to be acceptable for our reality. However, the

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**Table 1 – Demographic and clinical characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Re-evaluated</th>
<th>Spontaneous resolution of the infection</th>
<th>Persistent infection</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>45 (30 – 65)</td>
<td>46 (30 – 65)</td>
<td>44 (30 – 65)</td>
<td>47 (30 – 65)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Menarche</td>
<td>13 (9 – 19)</td>
<td>13 (9 – 19)</td>
<td>13 (9 – 18)</td>
<td>13 (10 – 19)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Post-menopause</td>
<td>24.9% (41/165)</td>
<td>30.6% (37/121)</td>
<td>26.9% (18/67)</td>
<td>35.2% (19/54)</td>
<td>0.01</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>20.3% (33/163)</td>
<td>21% (25/119)</td>
<td>23.1% (15/65)</td>
<td>18.5% (10/54)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Primiparous</td>
<td>30.7% (50/163)</td>
<td>31.1% (37/119)</td>
<td>29.2% (19/65)</td>
<td>33.3% (18/54)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Multiparous</td>
<td>49.1% (80/163)</td>
<td>47.9% (57/119)</td>
<td>47.7% (31/65)</td>
<td>48.2% (26/54)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Coitarche</td>
<td>19 (12 – 41)</td>
<td>19 (12 – 41)</td>
<td>18 (13 – 34)</td>
<td>19 (12 – 41)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Oral contraception</td>
<td>37.8% (59/156)</td>
<td>34.5% (39/113)</td>
<td>34.9% (22/63)</td>
<td>34% (17/50)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>30.6% (45/147)</td>
<td>32.7% (34/104)</td>
<td>40.4% (23/57)</td>
<td>23.4% (11/47)</td>
<td>n.s.</td>
</tr>
<tr>
<td>HIV infection</td>
<td>13.3% (22/165)</td>
<td>14.1% (17/121)</td>
<td>9% (6/67)</td>
<td>20.4% (11/54)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Precancerous lesions(^a)</td>
<td>13.2% (16/121)</td>
<td>1.5% (1/67)</td>
<td>27.8% (15/54)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>HSIL</td>
<td>1.7% (2/121)</td>
<td>0% (0/67)</td>
<td>3.7% (2/54)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean (maximum-minimum) or as frequency (n/total)

\(^a\) Comparative analysis of women with spontaneous resolution of the infection and women with persistent infection

\(^b\) Identified by cytology

\(^c\) Non-significant
test used (Cobas® Roche) does not differentiate between individual non-16/18 HPV types and we know that their isolated potential of malignancy is different. One of the strengths of our study is that we only included women aged 30 to 65, as it is recommended.12,34

These preliminary results also support the opinion that referring all women with isolated non-16/18 hrHPV infection to colposcopy might not be the best option.12 Nevertheless, additional studies are needed to evaluate individual risks of progression to precancerous and malignant lesions. Finally, these women may be reassured that the infection might resolve spontaneously, after making sure that they understand the importance of re-evaluation.

CONCLUSION
According to our results, re-evaluation with co-test one year after the diagnosis of isolated non-16/18 hrHPV cervical infection seems to be a reasonable approach in our reality, since 55.4% of these women showed spontaneous regression of this infection and only 1.7% developed HSIL, that were not confirmed in a biopsy specimen.

PROTECTION OF HUMANS AND ANIMALS
The authors declare that the procedures were followed according to the regulations established by the Clinical

REFERENCES


