Screening and management of high-risk human papillomavirus infections: lessons from pandemic

Rastreio e orientação de infeções por Papilomavírus Humano de Alto Risco: aprendizagens decorrentes da pandemia

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Abstract

Introduction: At the beginning of COVID-19 pandemic, the Central Regional Health Administration recommended that all women with a positive HPV test for types non 16 or 18 (HR-HPV) and reflex cytology ASC-US/LSIL, should repeat screening after 12 months, in primary healthcare.
Objective: Our main goal was to evaluate the impact of this orientation, searching for persistence or elimination of HPV infection, related risk factors, and secondarily, assessing the development of dysplasia.
Design: A nested case-control was performed (cases: HPV persistence; control: HPV elimination).
Setting and Participants: The initial cohort, included 215 women (HR-HPV/ASCUS or LSIL), referred to the Gynecologic department of Portuguese Institute of Oncology of Coimbra. They were screened from 27 of March 2019 to 30 March of 2020. The data were collected through medical records, in March 2022.
Main outcome measures: This sample was evaluated through two rounds. In the first round, 189 were sent back to primary healthcare and 26 older women remained at the department. At the second round, 51 were readmitted, based on a second positive HPV test. For analysis purposes, we divided our sample in three groups: the first with 97 women that repeated the HPV test at primary healthcare (92 missed the retest), the second with 77 women evaluated at the department (26 at first round and 51 at second round) and the third, by 111 that repeated a second HPV test.
Results: About 47% of those who repeated the HPV test at primary healthcare, cleared the infection. From the 77 evaluated at the department, only five cases had HSIL/CIN2. After 24 months of follow-up, about 50% have HR-HPV infection. Smoking habits and menopause were related with higher persistence.
Conclusion: For women with HR-HPV and ASCUS or LSIL reflex cytology, repeating HPV testing at 12 months, seems to be a safety measure.

Keywords: Human papillomavirus DNA tests; Atypical squamous cells of the cervix; Squamous intraepithelial lesions.

Resumo

Introdução: No início da pandemia COVID-19, a Administração Regional de Saúde do Centro recomendou que todas as mulheres com teste de HPV positivo para tipos não 16 ou 18 (HR-HPV) e citologia reflexa ASC-US/LSIL deveriam repetir rastreio ao fim de 12 meses, em cuidados de saúde primários.
Objetivo: O objetivo principal do trabalho é avaliar o impacto desta orientação. Primariamente pretende-se avaliar

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persistência e/ou eliminação da infeção por HPV, e identificar potencias fatores de risco relacionados e, secundariamente, avaliar o desenvolvimento de displasia.
Desenho: Foi realizado um caso-controle aninhado (casos: persistência de infeção por HPV; controlos: eliminação da infeção).
Local e participantes: A coorte inicial incluiu 215 mulheres (HR-HPV/ASCUS ou LSIL), rastreadas de 27 de março 2019 a 30 de março de 2020 e encaminhadas para o Serviço de Ginecologia do Instituto Português de Oncologia de Coimbra. Os dados foram obtidos durante o mês de Março de 2022, por consulta dos processos clínicos.
Métodos: Esta amostra foi avaliada através de duas rondas. Na primeira, 189 mulheres foram devolvidas aos cuidados de saúde primários e 26 com idades superiores a 50 anos permaneceram no serviço. Na segunda ronda, 51 foram readmitidas, por segundo teste de HPV positivo. A análise da coorte foi estratificada em três grupos: o primeiro com 97 mulheres que repetiram o teste de HPV em cuidados de saúde primários (92 faltaram), o segundo com 77 mulheres avaliadas no serviço (26 na primeira ronda e 51 na segunda ronda) e o terceiro, pelas 111 mulheres que repetiram um segundo teste de HPV.
Resultados: Aproximadamente 47% das mulheres que repetiram o teste de HPV nos cuidados de saúde primários eliminaram a infeção. Das 77 avaliadas no serviço, apenas cincos desenvolveram HSIL/CIN2. Ao fim de 24 meses de seguimento, aproximadamente 50% manteve infeção por HR-HPV. O hábito tabágico e as mulheres na menopausa, apresentaram maior risco de persistência.
Conclusão: A reavaliação de mulheres com infeção HR-HPV e citologia reflexa ASCUS ou LSIL, ao fim de 12 meses da colheita inicial, parece ser uma medida segura.

Palavras-chave: Teste de DNA do papilomavírus humano; Células escamosas atípicas de significado indeterminado; Lesão intraepitelial escamosa de baixo grau.

INTRODUCTION

Human papillomavirus (HPV) is the most prevalent sexually transmitted infection, although, regardless of genotype, most infections are transient[1]. However, persistence of high-risk HPV infection is associated with approximately 90% of cervical cancers (CC)[1]. Beyond HPV infection, other cofactors are required for the development of invasive disease. It has been described associations with smoking habits, long-term use of hormonal contraceptives, multiparity and immunosuppression[2].

Cervical cancer screening (CCS) guidelines were updated in 2017 by the Portuguese Ministry of Health and implemented in 2019 in the Central Region of Portugal. The cytological screening program was replaced by primary HPV testing, which is performed with clinical validated Cobas® HPV test, that detects 14 high risk oncogenic HPV types: HPV 16, HPV 18, and other HR-HPV (31/33/35/39/45/51/52/56/58/59/66/68). The 16/18 genotyping works as a triage test for direct colposcopy and pap cytology as a triage for other 12 HR-HPV types[3,4]. If reflex pap test has alterations of equal or greater than atypical squamous cells of undetermined significance (ASCUS), it has indication to colposcopy. All women aged 25 to 60 years, are screened every five years[3,4].

In 2020, the onset of COVID-19 pandemic led to a reorganization of healthcare, with direct impact on "non-priority" activity, which culminated in the suspension of screenings between March and July, in all Portuguese Regional Health Administrations (RHA). Therefore, compared to 2019, 55% fewer women were screened for CC[5,6].

At this stage, recommendations were drawn up by Central RHA to guide women with altered screening tests. According to these, women with positive HPV test for types other than 16 and 18 (HR-HPV) and reflex cytology ASC-US/ LSIL, should be addressed to repeat screening within one year in primary healthcare[5].

Thus, the **aim** of this study is to assess the impact of the guidance of women with HR-HPV positive/reflex cytology-ASCUS or LSIL, referred to the Gynaecology department of the Portuguese Institute of Oncology of Coimbra (IPOC), in the initial phase of the pandemic. We sought to assess the prevalence of transient infec-

tions and which risk factors are associated with the persistence or elimination, and secondarily, we sought to assess the prevalence of high grade squamous intraepithelial lesions -HSIL/CIN2+.

MATERIAL AND METHODS

We conducted a nested case-control study of a cohort of women with HR-HPV and ASCUS or LSIL reflex cytology. The initial cohort included 215 women, screened from 27 March 2019 to 30 March 2020 and referred to the Gynaecology department of IPOC. The data were obtained during the month of March 2022, by consulting the *Siima* platform and the hospital clinical files.

This sample was evaluated through two rounds. At first round, 26 women, older than 50 years, remained at the department and 189 were sent back to primary healthcare. The option to not return older women, was taken based on the higher risk of persistence and progression of HPV, at these ages[7]. At second round, 51 were readmitted, based on a second positive HPV test. (Figure 1)

Based on this, the cohort analysis was then stratified by three groups: 1) the first group comprised 189 women referred to repeat HPV test at primary healthcare. Ninety-two women were excluded for not having repeated the test, so this group included 97 women; (Figure 1); 2) the second group consisted of the 77 women referred to the department, 26 women admitted in a first round and 51 in a second round, after a second positive HPV test. The evaluation of this group allows the assessment of HSIL/CIN2+ prevalence and the identification of cofactors connected with HPV persistent; 3) finally, the third group, included those women who repeated the HPV test (97 at primary healthcare and 14 at the department). From this third group, 15 women readmitted because of a second positive HPV test, where retested at the department. Thereby, a total of 126 HPV tests were performed. This allows us to assess

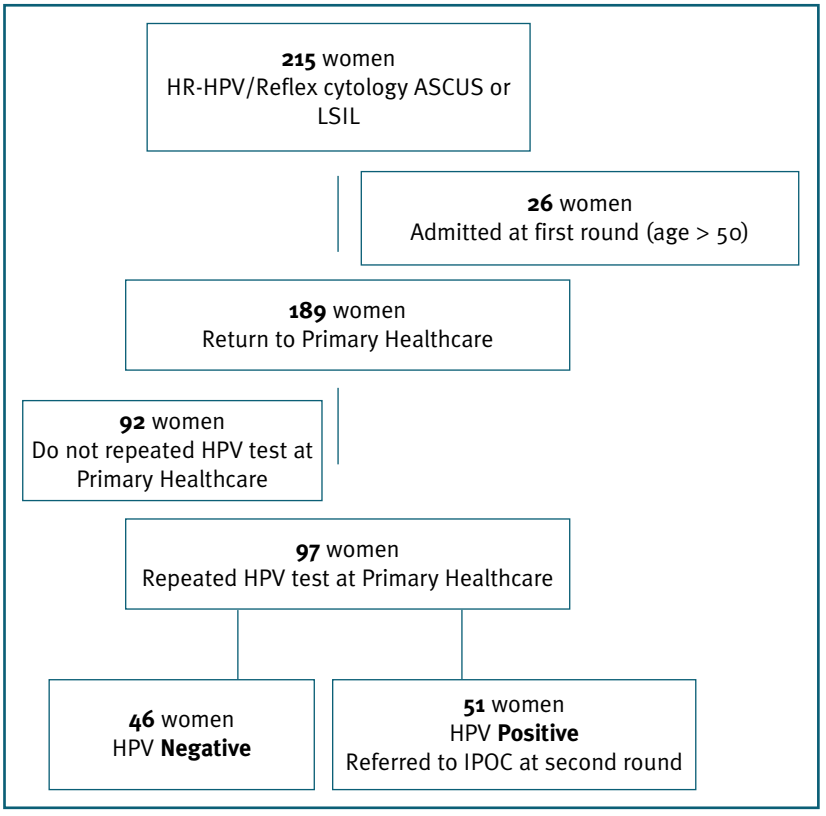

FIGURE 1. Study flow chart.

elimination/persistence rates and to identify potential associated cofactors.

Within the study, women with persistent HR-HPV infection were considered as cases and those who eliminated it were considered as controls.

The following variables were analyzed: age at diagnosis, contraceptive method, parity, smoking habits, menopause, HPV vaccination, history of lower genital tract treatments, first CCS, date of first collection, reflex cytology result, date of second collection, HPV typing (Cobas test®), referral to IPOC, colposcopy findings (according to 2011 International Federation of Cervical Pathology and Colposcopy nomenclature),[8] treatments performed, histology and time to HR-HPV clearance.

Statistical analysis was performed using the STATA program (version 13.1), with statistically significant results if $p \leq 0.05$ and 95% confidence level. Fisher's exact or chi-square test were applied to assess the relationship between nominal variables, and Student t-test or Wilcoxon's test were applied for comparison between measures of central tendency (according to the presence or absence of normal distribution). Adjustment for confounding factors (such as age, smoking, reproductive status, parity, contraception, or vaccination) was made by applying logistic regression. Kaplan-Meier curves were used to describe the distribution of elimination time and the log-rank test was used for comparison.

The study was approved by the ethics committee of IPOC and it has been conducted in accordance with the principles set forth in the Helsinki Declaration.

RESULTS

We initially assessed data from 215 women with HR--HPV infection and reflex cytology: ASCUS/LSIL. (Figure 1) Of those 97 women who repeated the HPV test at primary healthcare, approximately 47% cleared the infection. From this, five were older than 50 years, and all eliminated the infection. HPV clearance was not statistically associated with any of the analyzed cofactors, namely age, smoking habit, or vaccination status. (Table I) Within the cohort that cleared HR-HPV infection, the median time to clearance was 14.5 months [10.1-22.9].

In the second group of women (n:77), we found that, of the 51 women admitted to the gynecology department after a second positive HPV test, 94% had persistent infection with the same high-risk group, while 6% had other genotypes (one with HPV 16 and two with multiple infections). (Table II) In this group, it was possible to evaluate the histological findings (by

TABLE I. CHARACTERISTICS OF WOMEN WHO REPEATED HPV TEST AT PRIMARY HEALTHCARE.

N=97	HPV Elimination (n=46: 47.4%)	Second HPV Test Positive (n=51: 52.6%)	p *
Age at screening (p50)	44 (p5:25- p95:58)	40 (p5:25-p95:54)	0.08
Hormonal contraception	24 (52.2%)	33 (64.7%)	0.19
Menopause (n)	7 (15.2%)	3 (5.9%)	0.13
HPV Vaccination (yes)	5 (10.9%)	12 (24%)	0.09
Nulliparous	12 (26.1%)	15 (29.4%)	0.69
Multiparous (>1)	34 (73.9%)	36 (70.6%)	
Smoking habits	11 (23.9%)	14 (27.5%)	0.69
First Organized Screening	11 (23.9%)	15 (29.4%)	0.54
First Reflex Cytology			0.57
ASCUS	28 (60.9%)	31 (60.8%)	
LSIL	18 (39.1%)	20 (39.2%)	
Cervical treatments (yes)	1 (2.2%)	4 (8%)	0.20

*Univariate analysis

TABLE II. CHARACTERIZATION OF WOMEN EVALUATED AT GYNECOLOGY DEPARTMENT (N=77).

	Sample 1* 26 (33.7%)	Sample 2** 51 (66.3%)	p
Age at screening (p50)	58 (p5:55-p95:65)	40 (p5:25-p95:54)	**<0.001**
Age at first sexual intercourse (p50)	19 (p5:17-p95:27)	18 (p5:15-p95:22)	0.432
Second HPV test:	(n.a)		(n.a)
HR-HPV		48 (94.3%)	
HPV 16		1 (1.9%)	
HPV 16, 18 and HR-HPV		1 (1.9%)	
HPV 18, HR-HPV		1 (1.9%)	
Reflex Cytology (n:48)	(n.a)		(n.a)
ASCUS		28 (58.3%)	
LSIL		14 (29.2%)	
ASC-H		4 (8.3%)	
NILM		2 (4.2%)	
Colposcopy findings			
Normal	10 (47.6%)	13 (33.3%)	0.397
Grade 1	10 (47.6%)	20 (51.3%)	
Grade 2	1 (4.8%)	6 (15.4%)	
Treatment (n)	8 (30.8%)	18 (35.3%)	0.352
Type of treatment(n:26)			0.458
Loop Excision of Transformation zone (LETZ)	3 (37.5%)	9 (52.9%)	
Laser CO2 Vaporization	5 (62.5%)	9 (47.1%)	
ETZ (n:12)			**0.041**
No dysplasia	1 (33.3%)	0 (0.0%)	
LSIL	2 (66.7%)	4 (44.4%)	
HSIL (CIN2)	0 (0.0%)	5 (55.6%)	
HPV Elimination (29 submitted to HPV test)			0.042
Yes	9 (64.3%)	4 (26.7%)	
No	5 (35.7%)	11(73.3%)	
Follow-up at the department	17 (65.4%)	29 (56.9%)	0.202

*: Admitted at first round (age > 50 years, one positive HPV test)
**: Admitted at second round (second positive HPV test)
n.a.: not applicable

biopsy and/or loop excision of the transformation zone--LETZ) in 34 cases, with low-grade intraepithelial lesions identified in 74%, high grade intraepithelial lesions (HSIL/CIN2) in 15% and absence of dysplasia in the remaining. All HSIL/CIN2 lesions were identified in the group of women referred with a repeated positive HPV test. (Table II) We didn't find any statistically significant association between the severity of dysplastic lesions and the HPV genotype. Virus clearance was higher in women observed in the first round. (Table II) Per multivariate analysis, no significant association was observed with any of the risk factors analyzed (e.g., smoking habits, reproductive status, parity, vaccination status or reflex cytology result). Within the cohort referred to IPOC, the median time to clear the infection was 19.5 months [13.5-24.5].

Finally, when assessing the group of women who repeated the HPV test, we found that clearance and persistence were very similar. Per univariate analysis, it was possible to verify that, within the assessed risk factors, only age and menopausal status had a statistically significant difference (p<0.001). (Table III) When

TABLE III. EVALUATION OF WOMEN WHO REPEATED A SECOND HPV TEST.

N=111	HPV Elimination (n=55)	HPV Persistence (n=56)	p *
Age (p50)	44 (24-65)	57 (37-63)	**< 0.001**
Hormonal contraception	30 (50.0%)	3 (23.1%)	0.077
Menopause (n)	13 (21.0%)	9 (69.2%)	**<0.001**
HPV Vaccination (yes)	6 (9.7%)	0 (0%)	0.242
Nulliparous	14 (22.6%)	1 (7.7%)	0.519
Multiparous (>1)	30 (48.4%)	9 (69.2%)	
Smoking	13 (21.0%)	4 (30.8%)	0.442
First Reflex Cytology			0.584
ASCUS	33 (60.0%)	34 (60.8%)	
LSIL	22 (40.0%)	22 (39.2%)	
Treatment			0.924
Laser CO2 Vaporization	2 (66.7%)	3 (60%)	
LETZ	1 (33.3%)	2(40%)	
Histology			0.334
LSIL	1	2	

*Univariate analysis

adjusting for confounding factors such as smoking habits, parity, contraception or vaccination, no significant differences between groups could be identified. However, smoking was associated with a higher risk of persistence (OR: 2.62 (p: 0.244; 95% CI (0.52-13.28)), as well as menopausal status (OR: 6.06 (p: 0.156; 95% CI (0.50-72.97). We also found that, among women submitted to a third HPV test, about 75% had persistence, regardless of the treatment or other cofactors.

DISCUSSION

According to the literature, the transition to HPV screening program, although associated with more clinically relevant findings, entails a significant increase in referrals for colposcopy and an inherent higher burden of colposcopic units. Most of these referrals are mainly caused by HR-HPV infection with low grade reflex cytology (ASCUS and LSIL), rarely associated with high-grade lesions[9-10].

In evaluating a sample of women with HR-HPV and low-grade (ASCUS/LSIL) reflex cytology, we demonstrated that it was possible to avoid the referral of 46 women without compromising safety.

Recently, different societies have updated the CCS guidelines, based on an individualized risk stratification of developing HSIL/CIN3+ lesions ("equal risk, equal approach")[11].

The Australian National CCS Program, published in January 2022, is based on a partial genotyping HPV test (16/18 and others), every five years, as it happens with the Portuguese CCS program[12]. However, risk is stratified into three levels: low, intermediate, and high. The intermediate risk includes HR-HPV infection and low-grade reflex cytology (NILM, ASCUS or LSIL). These women repeated the test after 12 months. These guidelines were based on the study of 8425 women (NILM:5825; ASCUS/LSIL:2600) aged 25 to 74 years, in which a high prevalence of transient infections was observed[13]. This triage strategy was also adopted by other programs[14].

Like the Australian program, the recently updated Portuguese guidelines also mention the HSIL/CIN3+ risk. According to this consensus, women with HR--HPV and ASCUS or LSIL reflex cytology, have indication for colposcopy, based on the risk for HSIL/CIN3+, higher than 4%[15].

Through our study, we observed that smokers had almost three times the odds of having HPV persisten-

ce than non-smokers, and postmenopausal women had six times the odds of having HPV persistence than premenopausal women, as previously reported[7]. We also verified that only five cases of HSIL (CIN2) were detected, all in women referred for persistence (second HPV positive test) regardless of the initial result of reflex cytology. Also, Gage J. *et al.* had demonstrated that the risk of CIN2+ lesions is similar in the group of HR-HPV infections, despite the reflex cytology findings (logistic-Weibull model: 3-year CIN2+ risk: 15.7% (HPV/ASCUS) vs. 15.2% ((HPV/LSIL)), p = 0.5)[16].

Concerning the older women (age 50+), we observed that, despite higher persistence of HPV infection, this was not associated with high-grade lesions development in these age groups. Similarly, Plummer *et al,* found that, in 4504 women with HR-HPV infection and ASCUS/LSIL cytology, those aged 50 years or older had the highest persistence rate at 24 months (OR: 1.47 (1.11-1.94))[17]. Instead, Gage J. and collaborators, verified that among a cohort of 972,029 women aged 30-64, the rates of both prevalent and incident HPV infections declined dramatically by age, while the associated 5-year CIN3+ risk did not increase with age[18]. Therefore, based on this and on our results, we could suggest that there is no need to enact a different approach for older women.

Plummer *et al,* also found that regardless of age, when the HPV infection persisted at 12 months, is expected to continue at 18 and 24 months.[17] These outcomes are in accordance with our findings in the group of women referred to the institution, i.e., the rate of HPV clearance was lower in those who had a second HPV positive test.

The strengths we identified in our study are its simplicity of execution and the adjustment for potential confounding factors.

The limitations identified are the retrospective nature, the size of the sample and the inclusion of only one center. Another limitation is related to the group of women who did not repeat the test at primary healthcare – it represents a high proportion of missing data, compromising the effectiveness of our study. However, as a result of this research, they were recalled for repetition of the test in primary healthcare. Finally, it should be noted that the Cobas test® does not distinguish HR-HPV other than HPV16 or 18, so it is not possible to know whether a persistent positive HR-HPV test is a true persistence or reinfection with another HR-HPV genotypes.

With this study, we demonstrated that this approach would enable better resource management (unnecessary medical appointments, exams, and treatments) without losing the safety and quality of the care.

As a final remark, we consider that larger studies would be useful, to provide stronger and more reliable results, for the purpose of reassessing women with HR-HPV infection and reflex cytology ASCUS or LSIL at primary healthcare, 12 months after the initial sample.

REFERENCES

1. Centers for Disease Control and Prevention. Cancers Associated with Human Papillomavirus, United States – 2014-2018. USCS Data Brief, no. 26. Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services; 2021.

2. Gheit T. Mucosal and Cutaneous Human Papillomavirus Infections and Cancer Biology. Front Oncol. 9:355;2019.

3. Diário da República n.º 183/2017, Série II de 2017-09-21, páginas 20788 – 20789

4. Sociedade Portuguesa de Ginecologia – Secção Portuguesa de Colposcopia e Patologia Cervico-vulvovaginal; Consenso sobre infeção por HPV e neoplasia intraepitelial do colo vulva e vagina, 2014.

5. Programa Nacional Para As Doenças Oncológicas Avaliação E Monitorização Dos Rastreios Oncológicos Organizados De Base Populacional 2019/2020 – Portugal; Lisboa: Direção-Geral da Saúde, 2021.

6. Recomendações para realização de RCCU e orientação de mulheres com teste de rastreio do cancro do colo do útero alterado durante o período de pandemia COVID-19; Sociedade Portuguesa de Colposcopia e Patologia do Trato Genital Inferior, 2020

7. Wang YY, Kong LH, Liu Y, Wang S, Fan QB, Zhu L, et al. Retrospective analysis of cervical cancer and precancerous lesions in patients with atypical squamous cells of undetermined significance in China. Med (United States). 2019;98(49):e18239

8. Bornstein J, Bentley J, Bösze P, Girardi F, Haefner H, Menton M, et al. 2011 colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. Obstet Gynecol. 2012 Jul;120(1):166-72

9. Bonde J. et al. Human Papillomavirus Same Genotype persistence and Risk: A systematic review. J Low Genit Tract Dis 2021;25: 27-37

10. Dick, S., Vink, F.J., Heideman, D.A.M. et al. Risk-stratification of HPV-positive women with low-grade cytology by FAM19A4/miR124-2 methylation and HPV genotyping. Br J Cancer 126,259-264(2022)

11. Perkins R, et al. 2019 ASCCP Risk-Based Management Consensus Guidelines for Abnormal Cervical Cancer Screening Tests

and Cancer Precursors. Journal of Lower Genital Tract Disease 2020, Volume 24, Number 2

12. National Cervical Screening Program – Understanding the National Cervical Screening Program Management Pathway: A guide for healthcare providers, updated 2022

13. Machalek D. et al. Routine cervical screening by primary HPV testing: early findings in the renewed National Cervical Screening Program. Medical Journal of Australia 211(3);2019

14. Kaljouw S, Jansen EEL, Aitken CA, Harrijvan LM, Naber SK, de Kok IMCM. Reducing unnecessary referrals for colposcopy in hrHPV-positive women within the Dutch cervical cancer screening programme: A modelling study. Gynecol Oncol. 2021 Mar 1;160(3): 713-20

15. Pedro A, Pacheco A, Sousa R, Mendinhos G, Miranda M, Urzal C, et al. Orientações de Consenso para a Abordagem dos Resultados Alterados nos Testes de Rastreio do Cancro do Colo do Útero pela SPCPTGI. Acta Med Port. 2023 Apr 3;36(4):285-295. Portuguese. Jan 23

15. Gage G. et al New Mexico HPV Pap Registry Steering Committee. Risk Stratification Using Human Papillomavirus Testing among Women with Equivocally Abnormal Cytology: Results from a State-Wide Surveillance Program. Cancer Epidemiol Biomarkers Prev. 2016;25(1):36-42

16. Plummer M. et al A 2-Year Prospective Study of Human Papillomavirus Persistence among Women with a Cytological Diagnosis of Atypical Squamous Cells of Undetermined Significance or Low-Grade Squamous Intraepithelial Lesion. The Journal of Infectious Diseases 2007;195:1582-9

17. Gage JC, Katki HA, Schiffman M, Fetterman B, Poitras NE, Lorey T, et al. Age-stratified 5-year risks of cervical precancer among women with enrollment and newly detected HPV infection. Int J Cancer. 2015 Apr 1;136(7):1665-71.

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Formal analysis: Fernanda Santos
Writing – Original Draft: Fernanda Santos
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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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RECEIVED: 03/10/2022
ACCEPTED: 14/07/2023