Gynecology and Obstetrics Clinical Medicine 3 (2023) 22–29

Contents lists available at ScienceDirect

Gynecology and Obstetrics Clinical Medicine

journal homepage: www.keaipublishing.com/en/journals/gynecology-and-obstetrics-clinical-medicine

Review Article

Clinical significance of extended high-risk human papillomavirus genotyping and viral load in cervical cancer and precancerous lesions

Pingping Su [a], Jincheng Ma [a], Lirui Yu [a], Shuting Tang [a], Pengming Sun [a,b,c,*]

[a] Laboratory of Gynecologic Oncology, Department of Gynecology, Fujian Maternity and Child Health Hospital, College of Clinical Medicine for Obstetrics & Gynecology and Pediatrics, Fujian Medical University, Fuzhou, 350001, Fujian, China
[b] Fujian Key Laboratory of Women and Children's Critical Diseases Research, Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, 350001, China
[c] Fujian Clinical Research Center for Gynecological Oncology, Fujian Maternity and Child Health Hospital (Fujian Obstetrics and Gynecology Hospital), Fuzhou, 350001, Fujian, China

ARTICLE INFO

Keywords:
High-risk human papillomavirus
Cervical cancer
Genotype
Viral load
Cancer screening

ABSTRACT

Persistent infections with specific high-risk human papillomavirus (HR-HPV) strains are the leading cause of cervical cancer and precancerous lesions. HPV-16 and HPV-18 are associated with more than 70% of cervical cancer. However, with recent widespread vaccination efforts against cervical cancer, the infection rates of HPV-16 and HPV-18 have decreased across all age groups, while the infection rates of other HR-HPV strains have increased. The non-16/18 HR-HPV strains play an important role in cervical lesions. These strains can be identified with extended genotyping, and the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines recommended an HPV-based testing to assess the risk of cervical disease in patients. We reviewed and analyzed the clinical benefits of applying extended HR-HPV genotyping, which was published by the International Agency for Research on Cancer (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), to cervical cancer screening. This review concluded that cervical cancer screening needs to include extended HR-HPV genotyping. The examination of extended HR-HPV genotyping in cervical intraepithelial lesions and cervical cancers can help guide clinical practices.

1. Introduction

Cervical cancer is the fourth most common malignant tumor after breast cancer, colorectal cancer, and lung cancer, threating the health of women worldwide.[1] Persistent infections by specific high-risk human papillomavirus (HR-HPV) strains are the leading cause of cervical cancer and precancerous lesions.[2] The International Agency for Research on Cancer (IARC) published genotyping results of 14 HR-HPV strains: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Among them, HPV-16 is the most common type of HR-HPV followed by HPV-31 and HPV-18,[3,4] and more than 50% of cervical intraepithelial neoplasia grade 3 or higher (CIN3+) lesions are related to HPV-16 infection.[5] Moreover, HPV-16 and HPV-18 are reportedly related to more than 70% of cervical cancer cases; thus, the research on HPV-16 and HPV-18 strains is the most extensive.[6]

The type-specific HPV prevalence in women with and without cervical lesions in the World were gathered from specific databases created at the Institute Catalan Oncology (ICO) and the IARC were shown in Fig. 1 (Available from: www.hpvcentre.net). With the implementation of the global HPV vaccination program, the proportion of HPV-16/18 infections has gradually decreased, while that of infections with other high-risk genotypes, such as HPV-52 and HPV-58, has relatively increased.[7,8] In high-grade cervical lesions, HR-HPV genotypes, such as 31, 33, 52, and 58, are more common than 18.[9,10] A certain degree of inconsistency is

* Corresponding author. Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University, 18 Daoshan Road, Fuzhou, 350001, Fujian, China.
E-mail address: fmsun1975@fjmu.edu.cn (P. Sun).

Publishing services by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.gocm.2023.01.001
Received 27 November 2022; Received in revised form 7 January 2023; Accepted 18 January 2023
Available online 26 January 2023

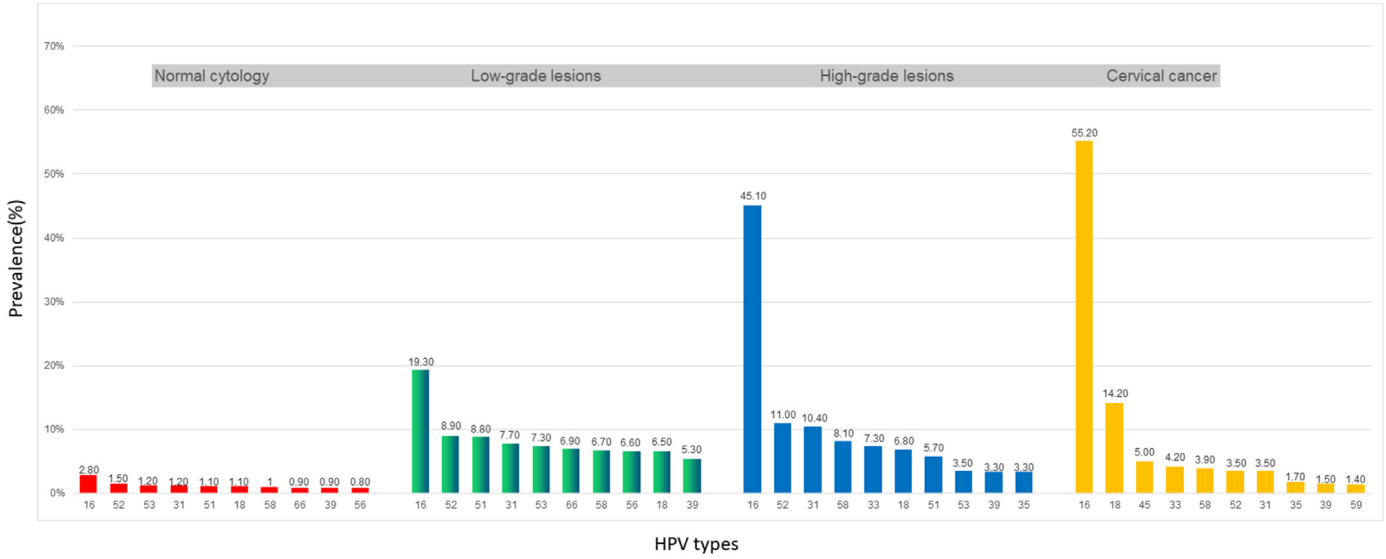

Fig. 1. Comparison of the ten most frequent human papillomavirus (HPV) oncogenic types in the World among women with and without cervical lesions. HPV-related statistics were gathered from specific databases created at the Institute Catalan Oncology (ICO) and the International Agency for Research on Cancer (IARC). Available from: www.hpvcentre.net.

associated with existing screening strategies, such as the sensitivity of cytological or HPV detection technology, may increase the risk of missed diagnosis as well as heavy burden on outpatients.[11] At the same time, long-term follow-up increases patient anxiety about cervical cancer, and therefore extended HR-HPV genotyping plays an important role in cervical cancer screening.[12] Consequently, risk evaluation, treatment, and prognosis of HR-HPV infections with these genotypes and further stratification of extended HR-HPV genotyping is needed. In this review, we analyzed the clinical benefits of applying extended HR-HPV genotyping to cervical cancer screening. Risk stratification based on extended HR-HPV genotyping will help guide future clinical work.

2. Cervical cancer screening needs to include extended HR-HPV genotyping

HR-HPV genotypes have different distribution patterns in different countries and research populations.[13,14] HPV-52, HPV-16, HPV-58, and HPV-18 are likely the most common HPV genotypes in Asian countries.[15] Type-specific HPV prevalence also varies in women with normal cervical cytology, precancerous cervical lesions and invasive cervical cancer in China. The HPV-related statistics were gathered from ICO/IARC were shown in Fig. 2. According to the distribution of the top 10 HPV genotypes associated with cervical cancer in China, released by the World Health Organization (WHO)/ICO (Global HPV Information Center), we found that the most common pathogenic HR-HPV genotypes in the Chinese population are HPV-16, HPV-52, HPV-58, HPV-33, HPV-18, and HPV-31. The widespread use of the HPV vaccine has changed the proportion of HPV-16 and HPV-18 infections in this population. Other HPV subtypes, such as HPV-31, HPV-33, HPV-52, and HPV-58, are more common than HPV-18 in high-grade cervical lesions.[16–18] In addition, HPV-52 and HPV-58 are also commonly occurring genotypes and have a strong correlation with the occurrence and development of cervical cancer.[19,20] Therefore, the expansion of HR-HPV genotyping is worthy of close attention. Based on our research on the Fujian population in China,

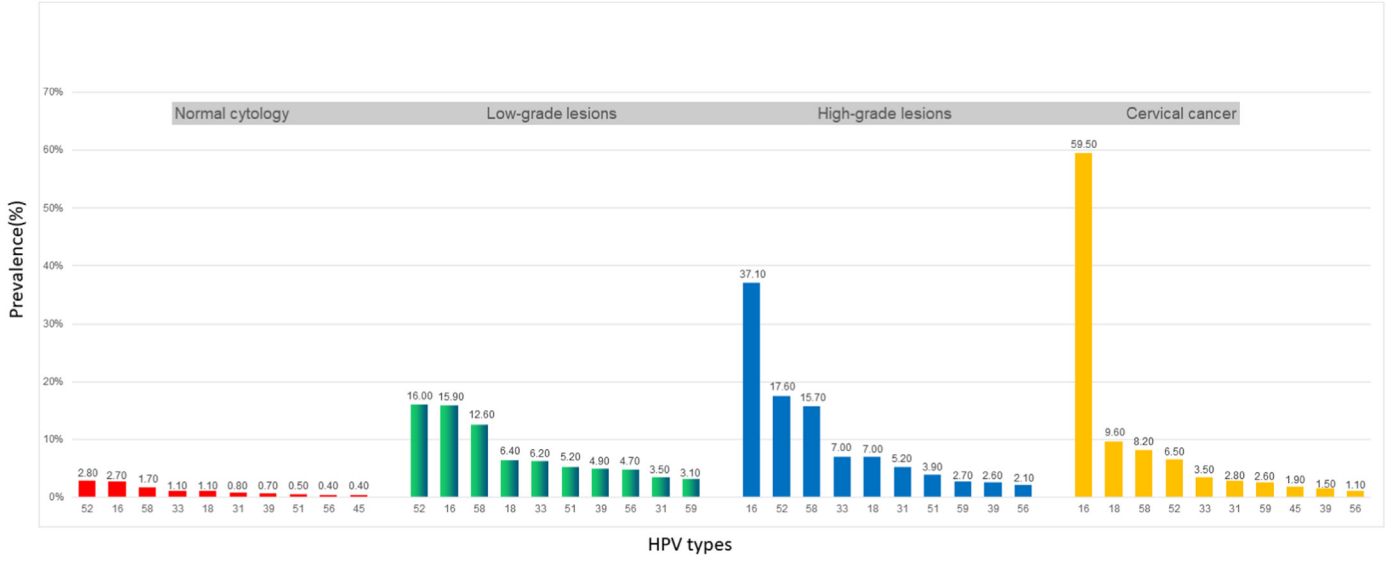

Fig. 2. Comparison of the ten most frequent HPV oncogenic types in China among women with and without cervical lesions. HPV-related statistics were available from ICO/IARC HPV Information Centre: www.hpvcentre.net.

Sun et al.[21] concluded that the cumulative risk of cervical lesions caused by the HR-HPV genotype infections varies in different grades of cervical lesions (low-grade cervical squamous intraepithelial lesions (LSIL), high-grade cervical squamous intraepithelial lesions (HSIL), and cervical cancer) (Fig. 3); further, the top five most common HPV infection genotypes in patients of different ages are different (Fig. 4). Here we summarize concrete data from several studies to address better risk prediction and clinical management by extended HR-HPV genotyping.

3. Risk assessment of cervical lesions based on HR-HPV infection in patients with abnormal cytology

In 2015, the Society of Gynecologic Oncology and the American Society for Colposcopy and Cervical Pathology (ASCCP) recommended primary HR-HPV screening in patients aged ≥25 years and cytological risk division for non-16/18 HR-HPV-positive women.[22] Therefore, clinical researchers have determined the effects of different HPV genotypes on cervical lesions through large-scale epidemiological research on cervical cancer and have established risk classifications based on HR-HPV genotypes; notably, HPV-31, HPV-33, HPV-52, and HPV-58 may lead to a higher probability of HSIL occurrence.[23,24] Ruan et al.[25] evaluated four cervical cancer screening strategies in 10,183 women in the Fujian Cervical Lesions Screening Cohort, considering CIN2+ and CIN3+ lesions as observation endpoints. They found that when HR-HPV was used for primary screening, HPV-positive women in group alpha 9 (HPV-16, -31, -33, -35, -52, and -58) were examined directly by colposcopy, while the HPV-positive women in the non-alpha 9 group were triaged by cytology. The sensitivity of the modified strategy to detect CIN3+ lesions was the highest at 89.85%, which may be a suitable strategy for cervical cancer screening in Chinese women. Based on a follow-up study of four European randomized controlled trials, Ronco et al.[26] suggested that HPV screening can improve the prevention of invasive cervical cancer by 60%–70% compared with cytology. Several important clinical trials on cervical cancer screening, such as ATHENA and Kaiser Permanente trials,[27,28] also proved the significance of HPV detection as a primary screening method. In 2019, the ASCCP guidelines approved the use of HPV genotyping for risk assessment. A "risk-based" treatment approach is proposed through either HPV screening alone or HPV and cytology co-test.[29] More frequent monitoring, colposcopy, and treatment are recommended for those with a CIN3+ risk of more than 4.0%, as determined by previous screening and current test results. For patients at lower risk, colposcopy can be postponed and follow-up interval may be increased; when the risk is low enough, they may return to routine screening[30] (Fig. 5). The research results shown in Table 1 support the use of specific HR-HPV genotyping to improve the triage management of cytology in ASC-US and LSIL. Nakamura et al.[43] found that among women with LSIL, determined by cytology, those who test negative for HPV-16/18/31/33/35/45/52/58 may not need immediate colposcopy and biopsy, leading to a reduction in the number of referrals for colposcopy by about 40%. This may greatly reduce the over treatment rate

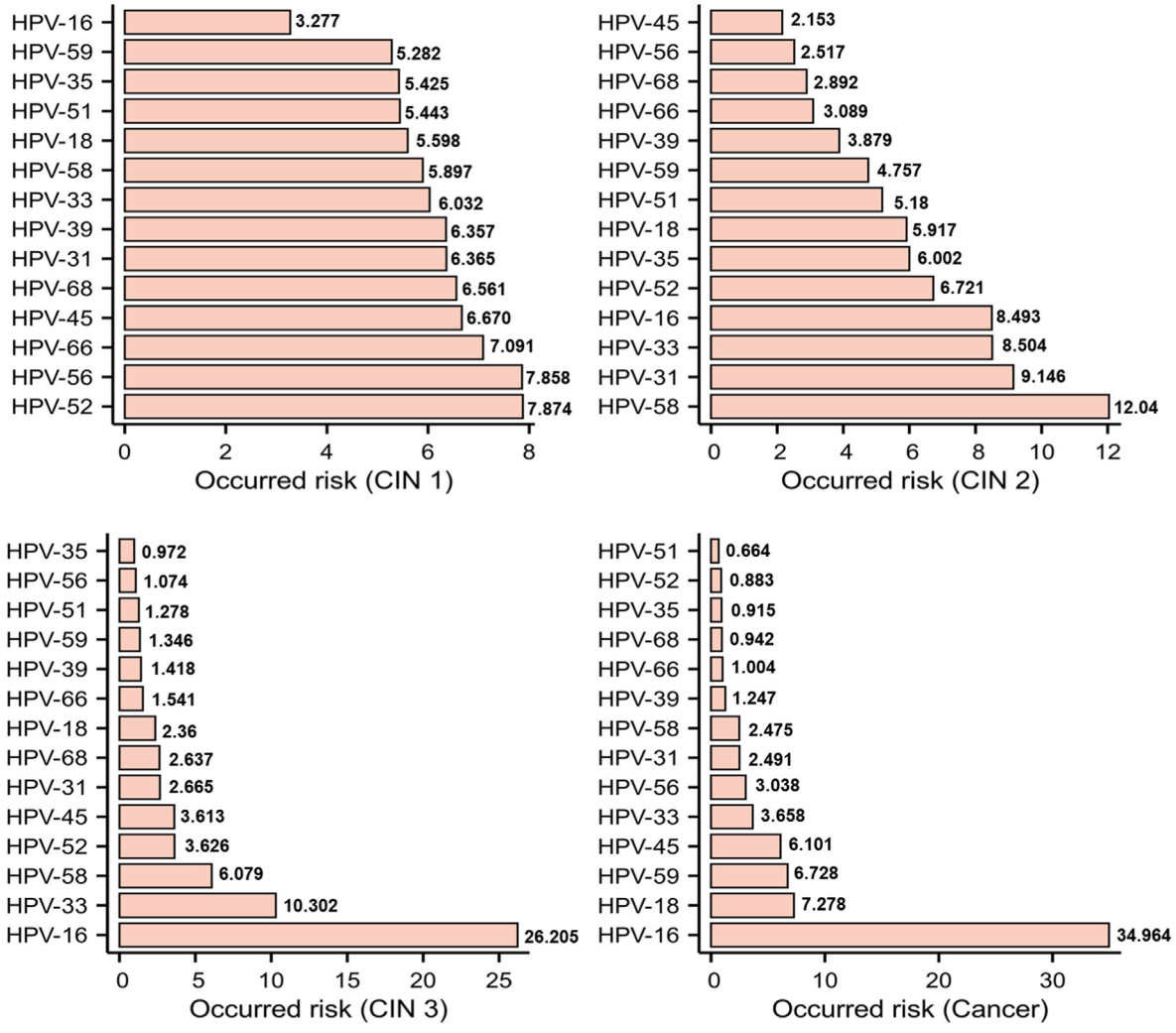

Fig. 3. Cumulative occurred risk of cervical lesions caused by different types of HR-HPV infection. Cumulative occurred risk of each HR-HPV genotype in patients with pathologically diagnosed (A) CIN1; (B) CIN2; (C) CIN3; and (D) invasive cervical cancer. Abbreviations: CIN, Cervical intraepithelial neoplasia.

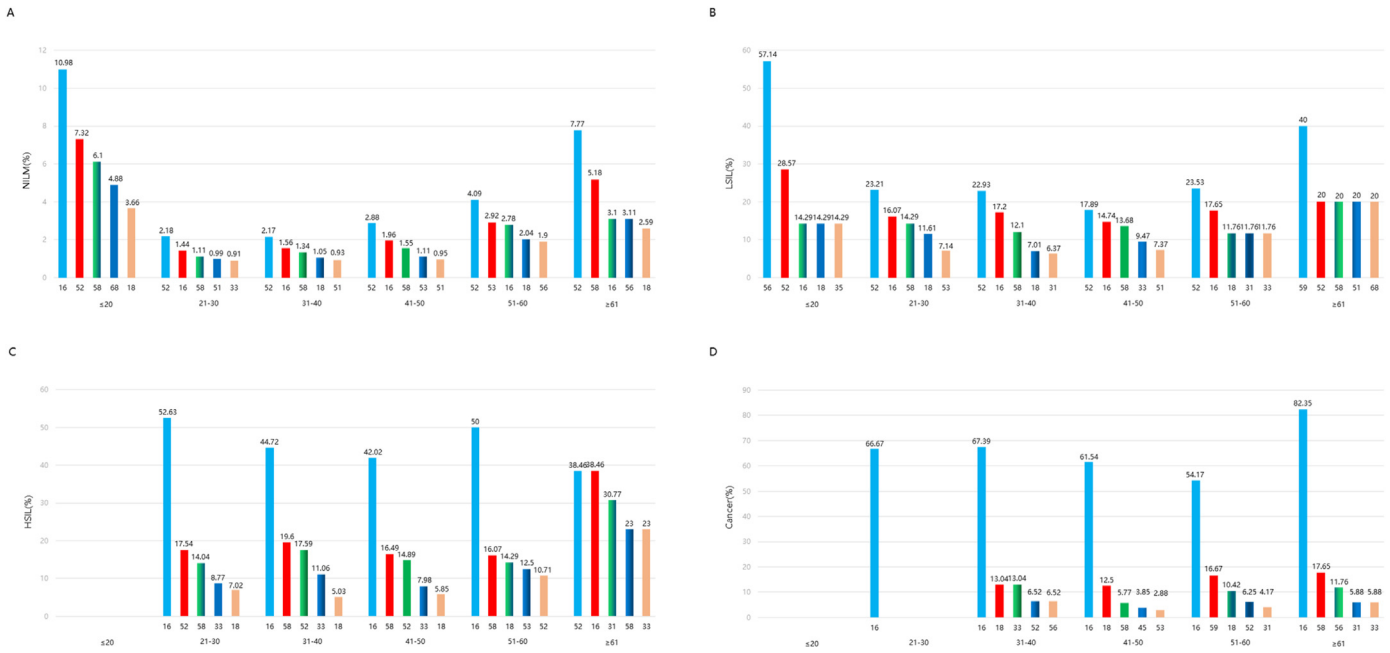

Fig. 4. The top five HPV genotypes in different ages of patients with pathologically diagnosed (A) NILM; (B) LSIL; (C) HSIL; and (D) invasive cervical cancer. Abbreviations: NILM, Negative for intraepithelial lesions or malignancies; LSILs, Low-grade cervical squamous intraepithelial lesions; HSILs, High-grade squamous intraepithelial lesions.

and improve the effectiveness of triage management. Therefore, primary screening and triage of cervical lesions through extended HR-HPV genotyping combined with cytology could precisely stratify the risk of cervical lesion, greatly improve the detection rate of cervical precancerous lesions, and thereby achieve more accurate colposcopy referral recommendations.

To date, most primary cervical screening guidelines do not recommend extended HR-HPV genotyping, although in some countries, HPV-16 and HPV-18 genotyping are used for cervical cancer triage. However, it is well known that extended HR-HPV genotyping helps to verify the persistence of specific HR-HPV types that are associated with a higher risk of developing CIN2+ lesions. In addition, HR-HPV genotyping information is needed to evaluate the epidemiological distribution of HR-HPV types in specific areas as well as the impact of local HPV vaccination activities, so as to generate scientific and epidemiological knowledge that would be helpful in formulating new cervical cancer screening policies.

4. Role of HR-HPV in cervical lesions after treatment

HPV-positivity is the strongest predictor of disease recurrence and progression. Zhang et al.[44] used the extended HR-HPV genotyping to predict residual and recurrent CIN2+ in patients after treatment. After a 4-year follow-up, the cumulative incidence risk and hazard ratio (HR) of persistent type-specific HR-HPV infections were the highest. Patients with both pre- and post-operative CIN2+ and CIN3+ lesions showed a higher proportion of HPV-16 infection, while the infection rate of HPV-52/58 was higher than that of HPV-18 both before and after operation. Interestingly, HPV-53 ranked fifth in terms of the proportion of post-operative CIN2+ and CIN3+ infections. An observational cohort study by Padalko et al.[45] on the potential etiological association between HPV-53 infection and HSIL development revealed that HPV-53 lacks carcinogenic potential. As multiple type infections are very common, specific HPV type infections may depend on the simultaneous existence of other HPV types. Therefore, further research is needed to determine the synergy between different HPV genotypes in multiple infections. With CIN2+ and CIN3+ as follow-up endpoints, specific HPV genotyping has a higher sensitivity (84.62%/89.28%) than cytological detection methods (60.42%/62.16%). The follow-up values of specific HR-HPV genotypes can provide reliable clinical reference for patients with CIN2+ lesions after operation.[44] Through a population-based retrospective cohort study, Dong et al.[46] analyzed the clinical benefits of applying extended HR-HPV genotyping to cervical cancer screening in China and concluded that the introduction of extended HR-HPV genotyping plans improved the early detection of CIN2+ lesions without increasing the cost, which may accelerate the elimination of cervical cancer in China and improve the triage strategy of ASCCP that was proposed in 2019.

5. Risk assessment of cervical lesions based on HR-HPV load

The relationship between HPV load and cervical lesions is still controversial. Hesselink et al.[47] found that the viral load of HPV-16/18/31/33 has no additive value to stratify the risk of CIN2+ or CIN3+ in a population-based cervical screening cohort. However, HR-HPV load in cervical lesions may be as important as persistent HPV infection.[48] Evaluating specific HR-HPV genotype load in initial cytological samples can be a tool for identifying disease progression in high-risk patients. The viral load of specific HR-HPV genotypes was positively correlated with abnormal cytology.[49] According to a study by Wu et al.,[50] the HPV-16 load increases with the progression of cervical lesion grade, which plays a dominant role in cervical cancer. While HPV-18 load was low in the precancerous stage, it showed an upward trend in cancer. Interestingly, the other HR-HPV genotypes showed an inverse trend than that of HPV-18.[50] Similar results were reported that the increase in HPV-16 load alone is associated with the risk of CIN2+.[51] For women with a normal cytological diagnosis, ASC-US, or LSIL, increase in HPV load was significantly correlated with the risk of CIN2/3 development.[52] The cumulative risk of CIN2/3 occurrence increased with an increase in one unit of HPV-31, HPV-35, HPV-52, and HPV-58 loads. This correlation was slightly significant for HPV-33 and HPV-45 but not others.[52] After following up with women aged ≥20 years in Fujian Province for more than four years, Dong et al.[53] confirmed the value of HR-HPV genotyping combined with quantitative detection in cervical lesion screening. They found that HPV-16/31/33/52/58 loads positively correlated with the severity of cervical lesions. The higher the viral load, the higher the risk of HSIL. Notably, HPV-18/45/56/59 and

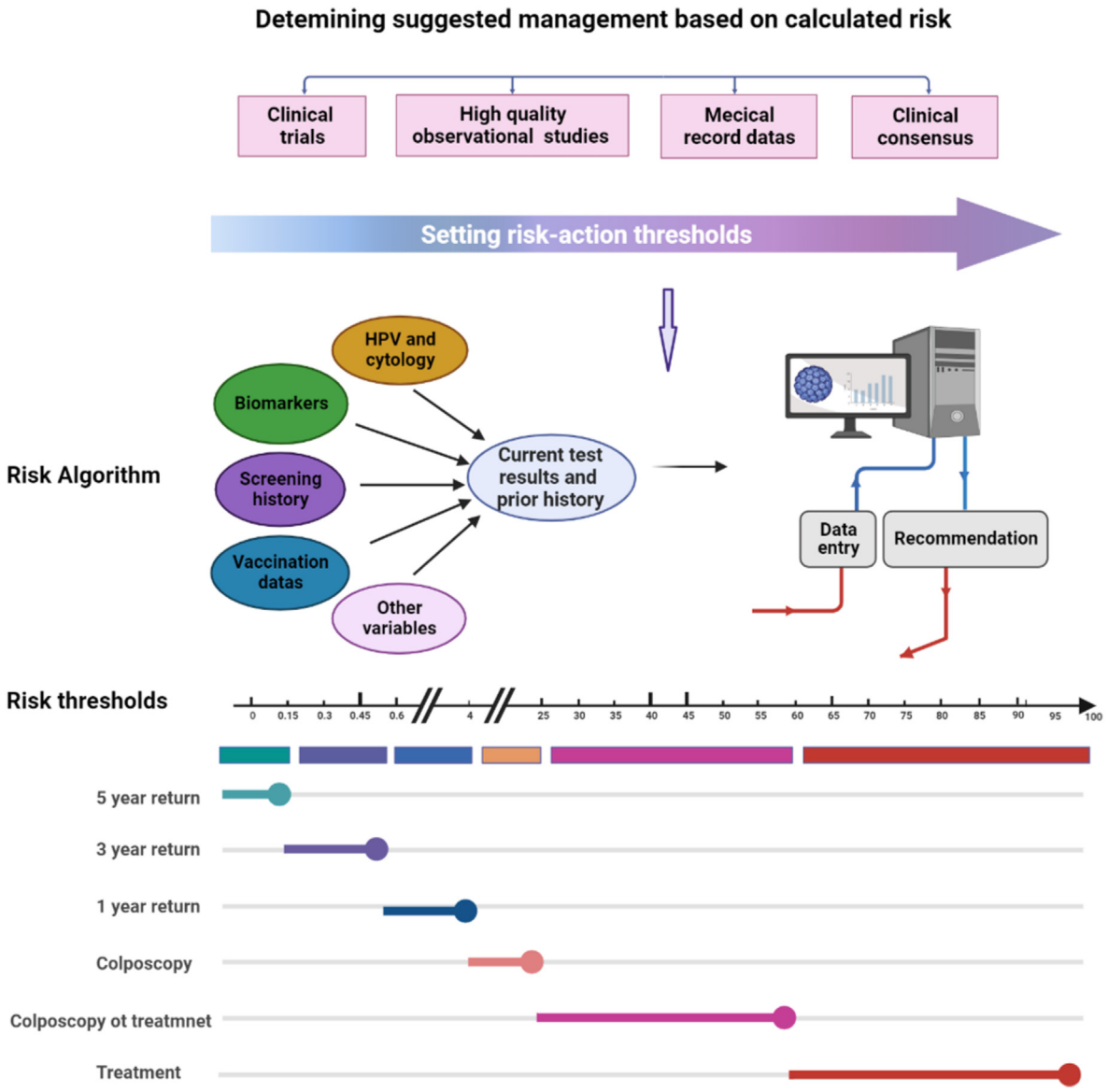

Fig. 5. Determining suggested management based on calculated risk. The risk-action thresholds were established through clinical trials, high quality observational studies, and medical record data. According to the current test results and prior history (HPV and cytology, biomarkers, screening history, vaccination data, and other variables), the risk of CIN2+/CIN3+ was calculated, a "risk-based" recommendation is proposed to determine the management on patients. Images were made in BioRender (Biorender.com).

other HR-HPV genotype loads were not found to be related to the grade of cervical lesions. These results support the use of viral load for triage of non-16/18 HR-HPV-positive women, especially alpha 9-positive women. This extended HR-HPV genotyping, combined with quantitative detection, is expected to become a new objective screening model independent of cytological triage. The optimal viral load of specific genotypes can be used as the clinical cutoff value for screening cervical lesions (≥HSIL), which could improve the specificity of screening, reduce missed diagnoses or misdiagnoses, and save medical resources.

6. Role of HR-HPV load in cervical lesions after treatment

Kang et al.[54] conducted a study to determine the prognostic significance of HPV load in cervical cancer patients (stage IB-IIA) undergoing radical hysterectomy; the results show that HPV load may not be helpful in predicting disease prognosis. However, by evaluating the predictive value of pre-operative HR-HPV loads in recurrent or residual cervical lesions after treatment of high-grade cervical lesions, Chen et al.[55] found that except for HPV-31/33, patients with high pre-operative HR-HPV-16/52/58 loads had significantly increased risk of residual cervical lesions. The possible reason for this result may be variation in the distribution of HPV genotypes in different regions and populations and likely effects of patient's age, fertility, the degree of cervical lesions, habits such as smoking, or treatment approach. The optimal cutoff value of HR-HPV load was set at 5.22 copies/10,000 cells (transformed by $\log^{10}$). The sensitivity of predicting residual lesions was 84.2% with a specificity of 77.0%, and it was inferred that these patients require more active treatment and follow-up strategies. However, considering that this is a single-center study, additional multicenter studies are necessary to continually assess the HPV loads for six months post-treatment. Nevertheless, this study has significance as a reference for the post-operative follow-up of patients. Thus, pre-operative HR-HPV loads are related to post-operative residual high-grade cervical lesions. This can be used to predict the occurrence of post-operative residual high-grade cervical lesions and further improve post-operative management. In low-resource areas lacking access to cytological screening methods, the application

Table 1
Studies of the extended HR-HPV genotyping on risk stratification for cervical cancer and precursors.

Reference	Country	Research design	Cases	Results	Follow-up
[31]	Sweden	prospective nested case-control	1226	The risk of cervical squamous cell carcinoma tripled within 7 years in patients who initially tested positive for non-16/18 HR-HPV than that for the HPV negative women.	7 years
[32]	Sweden	prospective multicenter study	5696	HPV-31 and HPV-33 positive women had a higher risk of CIN2+ than HPV-18 positive women.	4.1 years
[33]	USA	prospective multicenter study	27,037	HPV-16 and 31 have the highest risk of CIN2+ and CIN3+, and are most common in CIN2+ and CIN3+ cases. HPV-18, 33/58 and 52 constitute the intermediate risk range, and 45, 51, 35/39/68 and 56/59/66 have the lowest risk.	3 years
[34]	USA	prospective cohort study	167	The cytology in LSILs, patients with HPV-31, HPV-39, and HPV-52 infections had a high incidence of HSILs.	20–46 months
[35]	Korea	retrospective study	1102	HPV-31/33/35/45/52/58 genotypes are more likely to develop into HSIL or cervical cancer.	30 months
[5]	USA	prospective multicenter study	2807	When cytological examination results showed ASC-US or LSILs at baseline, HPV-16 had the highest risk of CIN2+, HPV-31, HPV-18, HPV-33/58, HPV-51, and HPV-52 were in the middle risk range, and HPV-35/39/68, HPV-45, and HPV-56/59/66 had the lowest risk of CIN2+.	3 years
[36]	China	retrospective study	902	Women with cytological LSIL combined with HR-HPV-16/18/31/33/52/58 positive results should be recommended for colposcopy.	3 years
[37]	Denmark	prospective cohort study	8656	The risk associated with HPV-16 was the highest to develop into CIN3+, followed by HPV-18, HPV-33, and HPV-31.	12 years
[38]	Turkey	retrospective study	179	HPV-31 has the highest risk of developing CIN2+ among non-16/18 HPV genotypes.	2 years
[39]	Thailand	cross-sectional study	5433	The detection rate of HSIL + could be increased by adding HPV-52/58 or HPV-31/52/58 genotyping to HPV-16/18 genotyping.	7 months
[40]	China	cross-sectional study	3997	HPV-16/18/33/52/58 showed a high sensitivity and specificity in detecting CIN2+ in ASCUS patients.	2 months
[41]	China	prospective observational study	19207	HPV-16/18/31/33/52/58 genotype positive patients have a high risk of CIN2+.	3 years
[42]	Japan	prospective cohort study	570	Women with cytological LSIL and histological CIN1-2 lesions, the cumulative probability of developing CIN 3 within 5 years was 20.5% for HPV16, 18, 31, 33, 35, 52, and 58.	39.1 months

of appropriate viral load cutoff values can be used as an effective triage tool to diagnose non-16/18 HR-HPV-positive women. Moreover, genotyping combined with viral load measurement would provide a more accurate reference value for the potential application of viral load measurement to improve the specificity of detection in cervical cancer screenings in the future.

7. Limitations of HR-HPV detection in cancer screening

Although the effectiveness of HR-HPV genotyping detection has been proven by clinical trials, it has its limitations. The systematic evaluation of 777 cervical cancer tissues from multiple cancer registries in the

United States revealed that HPV DNA was detected in 91% of cases; in other words, nearly 10% of cervical cancer patients are negative for HPV.[56] Moreover, approximately 37% of patients with cervical adenocarcinoma worldwide are HPV-negative.[56] Consistent with these results, a national multicenter retrospective study by Chen et al.[57] evaluating the correlation between HPV prevalence and cervical adenocarcinoma in China. The result shown that the infection rate of HPV in cervical adenocarcinoma (n=718) was 74.5%, indicating that that classical cervical adenocarcinoma is not always associated with HR-HPV. Moreover, HR-HPV status was mostly negative for special pathological types (minimal deviation adenocarcinoma, clear-cell adenocarcinoma, serous adenocarcinoma, endometrioid adenocarcinoma) of cervical adenocarcinoma. Their etiology is in dispute. The false negatives may arise due to factors such as latent HPV infection, histological misclassification, non-high risk HPV infection, disruption of the targeting fragment, and HPV testing methods.[58–62] Therefore, we strongly believe that HR-HPV infection is an important pathogenic factor, albeit not the only one, for cervical cancer. According to the management guidelines updated by ASCCP in 2019, the triage of screening patients was changed from results-based management to risk-based management.[63] The results of cytological examination still play an important role in the evaluation of risk models.

8. Conclusions

Cervical cancer screening strategies are changing from cytology screenings, cytology combined with HPV screenings to risk-based management strategies determined by HPV-based testing. Thus, HPV vaccination will greatly affect the performance of existing screening methods (which yield poor results based on cytological and non-genotyped HPV testing), and HPV genotyping needs to be expanded and incorporated into cervical cancer screenings in the future. Several studies have shown that HPV-positivity, which only indicates positive infection status, does not necessarily indicate pathological changes. We should predict risks by expanding the classifications and actively checking for pathological changes. Extended HR-HPV genotyping and quantitative detection may provide guidance for women with ASC-US and LSIL in treatment and follow-up decision-making. A series of clinical studies have shown that, due to the differences in ages and geographical distributions of HPV-infected patients, extended HR-HPV genotyping, quantitative detection, and risk stratification of HR-HPV strains could enable clinicians to identify the type of infection during a follow-up and fully assess the risks of CIN3+ or cervical cancer development. Risk stratification allows suitable recommendations for colposcopies and treatments to women with the highest risk of cervical diseases, while women with the lowest risk of pathological changes should be re-examined at short intervals; this would more effectively utilize health care resources, avoid missed diagnoses or misdiagnoses, and reduce patients' psychological anxiety. This review confirms the value of HR-HPV testing in cervical cancer screening while acknowledging the limitations of HPV testing and the shortcomings of research evidence. In the future we should implement further exploratory research, determine more accurate guidelines for clinical action thresholds, and strive to identify the indications for the application of various testing methods for different populations, especially in areas with inadequate cervical cancer screening conditions.

Authors' contributions

The study was designed by Pengming Sun. Pingping Su wrote the paper. Pengming Sun, Pingping Su, Jincheng Ma, Lirui Yu and Shuting Tang contributed to the supervision and reviewing the manuscript. The questions related to the accuracy or integrity of any part of the work are appropriately investigated by Pengming Sun, Pingping Su, Jincheng Ma, Lirui Yu and Shuting Tang. All authors approval of the final version to be published.

Funding

The work was supported by the Fund of National Key R&D Program of China (Grant no. 2021YFC2701205), the National Nature Science Foundation of China (Grant no. 82271658), and Major scientific research projects of young and middle-aged people of Fujian Provincial Health Commission (grant no. 2021ZQNZD011).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

References

1. Arbyn M, Weiderpass E, Bruni L, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Global Health*. 2020;8(2):e191–e203. https://doi.org/10.1016/S2214-109X(19)30482-6.
2. Sammarco ML, Del Riccio I, Tamburro M, et al. Type-specific persistence and associated risk factors of human papillomavirus infections in women living in central Italy. *Eur J Obstet Gynecol Reprod Biol*. 2013;168(2):222–226. https://doi.org/10.1016/j.ejogrb.2013.01.012.
3. Vintermyr OK, Andersland MS, Bjørge T, et al. Human papillomavirus type specific risk of progression and remission during long-term follow-up of equivocal and low-grade HPV-positive cervical smears. *Int J Cancer*. 2018;143(4):851–860. https://doi.org/10.1002/ijc.31390.
4. Wang SS, Zuna RE, Wentzensen N, et al. Human papillomavirus cofactors by disease progression and human papillomavirus types in the study to understand cervical cancer early endpoints and determinants. *Cancer Epidemiol Biomarkers Prev*. 2009;18(1):113–120. https://doi.org/10.1158/1055-9965.EPI-08-0591.
5. Wright TC, Stoler MH, Parvu V, et al. Risk detection for high-grade cervical disease using Onclarity HPV extended genotyping in women, ≥21 years of age, with ASC-US or LSIL cytology. *Gynecol Oncol*. 2019;154(2):360–367. https://doi.org/10.1016/j.ygyno.2019.05.012.
6. de Sanjose S, Quint WG, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *Lancet Oncol*. 2010;11(11):1048–1056. https://doi.org/10.1016/S1470-2045(10)70230-8.
7. Drolet M, É Bénard, Pérez N, et al. Population-level impact and herd effects following the introduction of human papillomavirus vaccination programmes: updated systematic review and meta-analysis. *Lancet*. 2019;394:497–509. https://doi.org/10.1016/S0140-6736(19)30298-3, 10197.
8. Ghosh T, VandeHaar MA, Rivera M, et al. High-risk HPV genotype distribution in HPV co-test specimens: study of a predominantly Midwestern population. *J Am Soc Cytopathol*. 2018;7(2):99–105. https://doi.org/10.1016/j.jasc.2017.09.007.
9. Monsonego J, Cox JT, Behrens C, et al. Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol Oncol*. 2015;137(1):47–54. https://doi.org/10.1016/j.ygyno.2015.01.551.
10. Schiffman M, Burk RD, Boyle S, et al. A study of genotyping for management of human papillomavirus-positive, cytology-negative cervical screening results. In: Patel R, ed. *J Clin Microbiol*. vol. 53. 2015:52–59. https://doi.org/10.1128/JCM.02116-14, 1.
11. Schiffman M, Kinney WK, Cheung LC, et al. Relative performance of HPV and cytology components of cotesting in cervical screening. *J Natl Cancer Inst*. 2018;110(5):501–508. https://doi.org/10.1093/jnci/djx225.
12. Cuzick J, Wheeler C. Need for expanded HPV genotyping for cervical screening. *Papillomavirus Res*. 2016;2:112–115. https://doi.org/10.1016/j.pvr.2016.05.004.
13. Li N, Franceschi S, Howell-Jones R, et al. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: variation by geographical region, histological type and year of publication. *Int J Cancer*. 2011;128(4):927–935. https://doi.org/10.1002/ijc.25396.
14. Zhu X, Wang Y, Lv Z, et al. Prevalence and genotype distribution of high-risk HPV infection among women in Beijing, China. *J Med Virol*. 2021;93(8):5103–5109. https://doi.org/10.1002/jmv.27013.
15. Crow JM. HPV: the global burden. *Nature*. 2012;488(7413):S2–S3. https://doi.org/10.1038/488S2a.
16. Adcock R, Cuzick J, Hunt WC, et al. Role of HPV genotype, multiple infections, and viral load on the risk of high-grade cervical neoplasia. *Cancer Epidemiol Biomarkers Prev*. 2019;28(11):1816–1824. https://doi.org/10.1158/1055-9965.EPI-19-0239.
17. Mateos Lindemann ML, Sánchez Calvo JM, Chacón de Antonio J, et al. Prevalence and distribution of high-risk genotypes of HPV in women with severe cervical lesions in madrid, Spain: importance of detecting genotype 16 and other high-risk genotypes. *Adv Prev Med*. 2011;2011:1–4. https://doi.org/10.4061/2011/269468.

18. Paz-Zulueta M, Álvarez-Paredes L, Rodríguez Díaz JC, et al. Prevalence of high-risk HPV genotypes, categorised by their quadrivalent and nine-valent HPV vaccination coverage, and the genotype association with high-grade lesions. *BMC Cancer*. 2018; 18(1):112. https://doi.org/10.1186/s12885-018-4033-2.
19. Xu HH, Wang K, Feng XJ, et al. Prevalence of human papillomavirus genotypes and relative risk of cervical cancer in China: a systematic review and meta-analysis. *Oncotarget*. 2018;9(20):15386–15397. https://doi.org/10.18632/oncotarget.24169.
20. Wang R, Guo X lei, GbeaA Wisman, et al. Nationwide prevalence of human papillomavirus infection and viral genotype distribution in 37 cities in China. *BMC Infect Dis*. 2015;15(1):257. https://doi.org/10.1186/s12879-015-0998-5.
21. Sun P, Song Y, Ruan G, et al. Clinical validation of the PCR-reverse dot blot human papillomavirus genotyping test in cervical lesions from Chinese women in the Fujian province: a hospital-based population study. *J Gynecol Oncol*. 2017;28(5):e50. https://doi.org/10.3802/jgo.2017.28.e50.
22. Huh WK, Ault KA, Chelmow D, et al. Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. *Gynecol Oncol*. 2015; 136(2):178–182. https://doi.org/10.1016/j.ygyno.2014.12.022.
23. Thomsen LT, Frederiksen K, Munk C, et al. Long-term risk of cervical intraepithelial neoplasia grade 3 or worse according to high-risk human papillomavirus genotype and semi-quantitative viral load among 33,288 women with normal cervical cytology: long-Term Risk of CIN3+ by HPV Genotype and viral load. *Int J Cancer*. 2015;137(1):193–203. https://doi.org/10.1002/ijc.29374.
24. Wheeler CM, Hunt WC, Cuzick J, et al. The influence of type-specific human papillomavirus infections on the detection of cervical precancer and cancer: a population-based study of opportunistic cervical screening in the United States. *Int J Cancer*. 2014;135(3):624–634. https://doi.org/10.1002/ijc.28605.
25. Ruan G, Song Y, Dong B, et al. Cervical cancer screening using the Cervista high-risk human papillomavirus test: opportunistic screening of a hospital-based population in Fujian province, China. *Cancer Manag Res*. 2018;10:3227–3235. https://doi.org/10.2147/CMAR.S169822.
26. Ronco G, Dillner J, Elfström KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. *Lancet*. 2014;383(9916):524–532. https://doi.org/10.1016/S0140-6736(13)62218-7.
27. Castle PE, Stoler MH, Wright TC, et al. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *Lancet Oncol*. 2011;12(9):880–890. https://doi.org/10.1016/S1470-2045(11)70188-7.
28. Katki HA, Kinney WK, Fetterman B, et al. Cervical cancer risk for 330,000 women undergoing concurrent HPV testing and cervical cytology in routine clinical practice at a large managed care organization. *Lancet Oncol*. 2011;12(7):663–672. https://doi.org/10.1016/S1470-2045(11)70145-0.
29. Perkins RB, Guido RS, Castle PE, et al. ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis*. 2019;24(2):102–131. https://doi.org/10.1097/LGT.0000000000000525, 2020.
30. Egemen D, Cheung LC, Chen X, et al. Risk estimates supporting the 2019 ASCCP risk-based management consensus guidelines. *J Low Genit Tract Dis*. 2020;24(2):132–143. https://doi.org/10.1097/LGT.0000000000000529.
31. Sundström K, Eloranta S, Sparén P, et al. Prospective study of human papillomavirus (HPV) types, HPV persistence, and risk of squamous cell carcinoma of the cervix. *Cancer Epidemiol Biomarkers Prev*. 2010;19(10):2469–2478. https://doi.org/10.1158/1055-9965.EPI-10-0424.
32. Naucler P, Ryd W, Törnberg S, et al. HPV type-specific risks of high-grade CIN during 4 years of follow-up: a population-based prospective study. *Br J Cancer*. 2007;97(1): 129–132. https://doi.org/10.1038/sj.bjc.6603843.
33. Stoler MH, Wright TC, Parvu V, et al. Stratified risk of high-grade cervical disease using onclarity HPV extended genotyping in women, ≥25 years of age, with NILM cytology. *Gynecol Oncol*. 2019;153(1):26–33. https://doi.org/10.1016/j.ygyno.2018.12.024.
34. Lyons YA, Kamat AA, Zhou H, et al. Non-16/18 high-risk HPV infection predicts disease persistence and progression in women with an initial interpretation of LSIL: non-16/18 HPV Predicts Disease Progression. *Cancer Cytopathol*. 2015;123(7): 435–442. https://doi.org/10.1002/cncy.21549.
35. Sung YE, Ki EY, Lee YS, et al. Can human papillomavirus (HPV) genotyping classify non-16/18 high-risk HPV infection by risk stratification? *J Gynecol Oncol*. 2016; 27(6):e56. https://doi.org/10.3802/jgo.2016.27.e56.
36. Xue H, Gao H, Zheng J, et al. Use of extended HR-HPV genotyping in improving the triage strategy of 2019 ASCCP recommendations in women with positive HR-HPV diagnosis and simultaneous LSIL cytology results. *J Cancer*. 2021;12(14):4332–4340. https://doi.org/10.7150/jca.55826.
37. Kjaer SK, Frederiksen K, Munk C, et al. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*. 2010;102(19):1478–1488. https://doi.org/10.1093/jnci/djq356.
38. Karaca İ, Öztürk M, Comba C, et al. Does the 'equal management of equal risks' model cause overtreatment in patients with positive cervical cytology results for ASCUS/non-HPV16/18 oncogenic types? *Diagn Cytopathol*. 2019;47(2):105–109. https://doi.org/10.1002/dc.24029.
39. Khunamornpong S, Settakorn J, Sukpan K, et al. Genotyping for human papillomavirus (HPV) 16/18/52/58 has a higher performance than HPV16/18 genotyping in triaging women with positive high-risk HPV test in northern Thailand. *PLoS One*. 2016;11(6), e0158184. https://doi.org/10.1371/journal.pone.0158184, 2016 Jun 23.
40. Guo Z, Jia MM, Chen Q, et al. Performance of different combination models of high-risk HPV genotyping in triaging Chinese women with atypical squamous cells of undetermined significance. *Front Oncol*. 2019;9:202. https://doi.org/10.3389/fonc.2019.00202.
41. Xu H, Lin A, Shao X, et al. Diagnostic accuracy of high-risk HPV genotyping in women with high-grade cervical lesions: evidence for improving the cervical cancer screening strategy in China. *Oncotarget*. 2016;7(50):83775–83783. https://doi.org/10.18632/oncotarget.11959.
42. Matsumoto K, Oki A, Furuta R, et al. Predicting the progression of cervical precursor lesions by human papillomavirus genotyping: a prospective cohort study. *Int J Cancer*. 2011;128(12):2898–2910. https://doi.org/10.1002/ijc.25630.
43. Nakamura Y, Matsumoto K, Satoh T, et al. HPV genotyping for triage of women with abnormal cervical cancer screening results: a multicenter prospective study. *Int J Clin Oncol*. 2015;20(5):974–981. https://doi.org/10.1007/s10147-015-0789-4.
44. Zhang Q, Dong B, Chen L, et al. Evaluation of PCR-reverse dot blot human papillomavirus genotyping test in predicting residual/recurrent CIN 2+ in posttreatment patients in China. *Cancer Manag Res*. 2020;12:2369–2379. https://doi.org/10.2147/CMAR.S237490.
45. Padalko E, Ali-Risasi C, Van Renterghem L, et al. Evaluation of the clinical significance of human papillomavirus (HPV) 53. *Eur J Obstet Gynecol Reprod Biol*. 2015;191:7–9. https://doi.org/10.1016/j.ejogrb.2015.04.004.
46. Dong B, Zou H, Mao X, et al. Effect of introducing human papillomavirus genotyping into real-world screening on cervical cancer screening in China: a retrospective population-based cohort study. *Ther Adv Med Oncol*. 2021;13, 175883592110109. https://doi.org/10.1177/17588359211010939.
47. Hesselink AT, Berkhof J, Heideman DAM, et al. High-risk human papillomavirus DNA load in a population-based cervical screening cohort in relation to the detection of high-grade cervical intraepithelial neoplasia and cervical cancer. *Int J Cancer*. 2009;124(2):381–386. https://doi.org/10.1002/ijc.23940.
48. Adcock R, Cuzick J, Hunt WC, et al. Role of HPV genotype, multiple infections, and viral load on the risk of high-grade cervical neoplasia. *Cancer Epidemiol Biomarkers Prev*. 2019;28(11):1816–1824. https://doi.org/10.1158/1055-9965.EPI-19-0239.
49. Dong L, Wang MZ, Zhao X lian, et al. Human papillomavirus viral load as a useful triage tool for non-16/18 high-risk human papillomavirus positive women: a prospective screening cohort study. *Gynecol Oncol*. 2018;148(1):103–110. https://doi.org/10.1016/j.ygyno.2017.11.016.
50. Wu Z, Qin Y, Yu L, et al. Association between human papillomavirus (HPV) 16, HPV18, and other HR-HPV viral load and the histological classification of cervical lesions: results from a large-scale cross-sectional study: HR-HPV Viral Load and Cervical Lesions. *J Med Virol*. 2017;89(3):535–541. https://doi.org/10.1002/jmv.24645.
51. Tao X, Austin RM, Yu T, et al. Risk stratification for cervical neoplasia using extended high-risk HPV genotyping in women with ASC-US cytology: a large retrospective study from China. *Cancer Cytopathol*. 2022;130(4):248–258. https://doi.org/10.1002/cncy.22536.
52. Fu Xi L, Schiffman M, Ke Y, et al. Type-dependent association between risk of cervical intraepithelial neoplasia and viral load of oncogenic human papillomavirus types other than types 16 and 18. *Int J Cancer*. 2017;140(8):1747–1756. https://doi.org/10.1002/ijc.30594.
53. Dong B, Sun P, Ruan G, et al. Type-specific high-risk human papillomavirus viral load as a viable triage indicator for high-grade squamous intraepithelial lesion: a nested case- control study. *Cancer Manag Res*. 2018;10:4839–4851. https://doi.org/10.2147/CMAR.S179724. Published 2018 Oct 23.
54. Kang WD, Kim CH, Cho MK, et al. HPV-18 is a poor prognostic factor, unlike the HPV viral load, in patients with stage IB–IIA cervical cancer undergoing radical hysterectomy. *Gynecol Oncol*. 2011;121(3):546–550. https://doi.org/10.1016/j.ygyno.2011.01.015.
55. Chen L, Dong B, Zhang Q, et al. HR-HPV viral load quality detection provide more accurate prediction for residual lesions after treatment: a prospective cohort study in patients with high-grade squamous lesions or worse. *Med Oncol*. 2020;37(5):37. https://doi.org/10.1007/s12032-020-01363-z.
56. Flanagan MB. Primary high-risk human papillomavirus testing for cervical cancer screening in the United States: is it time? *Arch Pathol Lab Med*. 2018;142(6):688–692. https://doi.org/10.5858/arpa.2018-0001-RA.
57. Chen W, Molijn A, Enqi W, et al. The variable clinicopathological categories and role of human papillomavirus in cervical adenocarcinoma: a hospital based nation-wide multi-center retrospective study across China: variable clinicopathological categories. *Int J Cancer*. 2016;139(12):2687–2697. https://doi.org/10.1002/ijc.30401.
58. Arezzo F, Cormio G, Loizzi V, et al. HPV-negative cervical cancer: a narrative review. *Diagnostics*. 2021;11(6):952. https://doi.org/10.3390/diagnostics11060952.
59. Rodríguez-Carunchio L, Soveral I, Steenbergen RD, et al. HPV-negative carcinoma of the uterine cervix: a distinct type of cervical cancer with poor prognosis. *BJOG*. 2015; 122(1):119–127. https://doi.org/10.1111/1471-0528.13071.
60. Barreto CL, Martins DBG, de Lima Filho JL, et al. Detection of Human Papillomavirus in biopsies of patients with cervical cancer, and its association with prognosis. *Arch Gynecol Obstet*. 2013;288(3):643–648. https://doi.org/10.1007/s00404-013-2803-2.
61. Villa LL, Denny L. CHAPTER 7 Methods for detection of HPV infection and its clinical utility. *Int J Gynaecol Obstet*. 2006;94:S71–S80. https://doi.org/10.1016/S0020-7292(07)60013-7.
62. Alemany L, Pérez C, Tous S, et al. Human papillomavirus genotype distribution in cervical cancer cases in Spain. Implications for prevention. *Gynecol Oncol*. 2012; 124(3):512–517. https://doi.org/10.1016/j.ygyno.2011.11.024.
63. Cheung LC, Egemen D, Chen X, et al. ASCCP risk-based management consensus guidelines: methods for risk estimation, recommended management, and validation. *J Low Genit Tract Dis*. 2019;24(2):90–101. https://doi.org/10.1097/LGT.0000000000000528, 2020.