

# Comprehensive overview of genotype distribution and prevalence of human papillomavirus in cervical lesions

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## ABSTRACT

Across cervical squamous and glandular lesions, a spectrum of human papillomavirus (HPV) genotypes has been identified. This review aims to provide a comprehensive summary detailing the distribution and profile of HPV genotypes detected in cervical lesions, leveraging insights from histological and cytological findings. High-risk HPV (HR-HPV) genotypes exhibit varying degrees of oncogenic potential, with HPV16 and HPV18 identified as the most prevalent and oncogenic types. The distribution of HR-HPV genotypes varies among different degrees of the cervical lesions and varies between squamous and glandular neoplasia. HPV16 is predominantly associated with severe lesions (precancers and carcinomas), while HPV18 demonstrates a significantly higher prevalence in endocervical as compared with squamous neoplasia. The distribution of HR-HPV in severe squamous lesions is complex, involving many HR-HPV genotypes in addition to HPV16, while the distribution of HR-HPV genotypes in endocervical glandular lesions is mainly limited in HPV18 and HPV16.

Large datasets from China have identified the three most common HR-HPV genotypes in this population as stratified by diagnostic category: HPV52, HPV16, HPV58 in histologically negative cases and cervical intraepithelial neoplasia 1 (CIN1); HPV16, HPV52, HPV58 in CIN2/3; HPV16, HPV58, HPV52 or HPV18 in squamous cell carcinoma (SCC); HPV16, HPV18 and HPV52 in endocervical adenocarcinoma in situ (AIS), invasive adenocarcinoma, as well as mixed squamous and glandular lesions. HPV33 is the fourth most common HPV type in CIN2/3 and SCC, while HPV45 occurs more commonly in AIS and adenocarcinoma, compared with squamous lesions. The prevalence and distribution of multiple HR-HPV coinfections vary across different cervical diseases. The clinical significance and pathogenesis of these multiple HR-HPV infections remain uncertain, although recent two large studies demonstrate that multiple HR-HPV infections are not associated with cumulatively higher risk of high-grade cervical squamous lesion development, suggesting competitive and/or cooperative interactions among HPV genotypes. Extensive HPV genotyping aids in risk assessment and optimising clinical approaches for women with mild abnormalities in Pap cytology. Women with atypical squamous cells of undetermined significance (ASC-US) and low-grade squamous intraepithelial lesion (LSIL) Pap test results and with the infection of some HR-HPV genotypes carry a very

low risk of high-grade cervical lesions. HPV genotyping can allow for risk stratification and triage optimisation for these HR-HPV-positive women. Women with atypical glandular cell (AGC) Pap test results showed a specific HPV genotyping pattern and extended HPV genotyping may be helpful for the clinical management of AGCs. Continual advancements in clinical guidelines integrating extended genotyping would increase diagnostic accuracy and refine strategies in clinical management.

Most cervical cancers have a defined aetiology that can be prevented and treated at an early stage. Persistent infection with high-risk human papillomavirus (HR-HPV) is considered a prerequisite for the development of cervical precancerous lesions and cervical carcinoma.<sup>1 2</sup> Among over 200 identifiable HPV genotypes, 15 are designated as high risk, including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82. Three genotypes are designated as probable high risk: HPV26, 53 and 66. Twelve additional genotypes including HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108, are designated as low risk.<sup>3</sup> Most literature acknowledges 18 HR-HPV types. Of these, HPV16 and HPV18 demonstrate the most carcinogenicity and contribute to more than 70% of cervical cancers and precancerous lesions.<sup>4</sup> Commercially available HPV vaccines, including the 2/4-valent vaccine (HPV16/18 plus HPV6/11) and the 9-valent vaccine (HPV16/18/31/33/45/52/58 plus HPV6/11), mainly prevent certain HPV genotype-associated infections, precancers and cancers.<sup>5</sup> Other HR-HPV genotypes have not been well studied for their oncogenic potential due to low prevalence and pooled HPV testing. Generally, the prevalence and genotype distribution of HPV varies in different regions, populations, socioeconomic statuses, ages and underlying disease states. Concomitant multiple ( $\geq 2$ ) HR-HPV infections are also not uncommon in cervical lesions.<sup>6-8</sup> The scope of this review is to provide a comprehensive information on

HPV genotyping, HR-HPV prevalence and HPV genotype distribution in cervical lesions, as well as to establish an objective foundational knowledge for HPV-based cervical cancer screening and evaluate the influence of HPV vaccination.

## 1. HR-HPV TESTING AND GENOTYPING METHODS

HPV testing in the clinical context refers to detecting a group of highly carcinogenic HPV genotypes, including 14 high-risk HPV types: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 66. Of these, HPV16/18 are the highest-risk genotypes, and distinguishing them from other HR-HPV genotypes is recommended by 2021 WHO guidelines.<sup>9</sup> HPV testing in cervical cancer screening has gone through a shift from reflex testing of cytological diagnoses of atypical squamous cells of undetermined significance (ASC-US), co-testing with cytology and now to HPV testing as a primary screen.<sup>10</sup> HPV testing has quickly become an essential component in most laboratories and clinical practices.

Currently in the USA, four HPV tests approved by the Food and Drug Administration (FDA) are commonly used: (1) Hybrid Capture 2 test (approved in 2003), which detects the presence of 13 HR-HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) without specific genotyping<sup>11</sup>; (2) Roche Cobas HPV assay (approved in 2011), which detects HPV16 and 18 individually, as well as a pool of 12 other HR-HPV genotypes; (3) Aptima HPV assay (approved in 2012), which detects the E6 and E7 mRNA transcripts of 14 HR-HPV types and can be used as an adjunct with concurrent Pap test for women aged 30 years and older as a reflex test for ASC-US cytology results, and with the Aptima HPV16/18/45 genotype assay to optimise the detection of adenocarcinoma (ADC); (4) Onclarity HPV assay by Becton Dickinson (approved in 2018), which detects HPV genotypes 16, 18, 45, 31, 51 and 52 individually, and a combination of 33+58, 35+39+68 and 56+59+66.<sup>12</sup> Of above assays, the Roche Cobas HPV assay and Onclarity HPV assay have been approved by FDA for primary HPV cervical cancer screening. There exists a trend from no HR-HPV genotyping, to partial and expanded HR-HPV genotyping for FDA-approved HPV testing assays.

In addition to the HPV assays made by the USA and European countries and used in China, there are many types of China FDA-approved HPV testing assays from different companies in China. It is estimated there are about 40 types of HPV full genotyping products, about 40 types of products with partial or no genotyping of HPV, and 11 types of products that only genotype HPV16/18. The five representative full HPV genotyping products routinely used in clinical practice in China include: (1) HPV Genotyping Kit for 23 Types (Yaneng BIOscience Co, Shenzhen, China), which includes 17 HR-HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 6 low-risk HPV (LR-HPV) genotypes (HPV6, 11, 42, 43, 81 and 83); (2) 21 HPV GenoArray

Diagnostic Kit (Hyribio Co, Guangdong, China) which includes testing for 15 HR-HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 53 and 66) and 6 LR-HPV genotypes (HPV6, 11, 42, 43, 44 and CP8304); (3) HPV 21 Genotyping Real Time PCR Kit (Jiangsu BioPerfectus Co, Jiangsu, China) which includes testing for 18 HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 26, 53, 66, 73 and 82) and 3 LR-HPV genotypes (HPV6, 11 and 81); (4) TellgenplexHPV27 genotyping assay (Tellgen Co, Shanghai, China) which includes testing for 17 HR-HPV genotypes (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 82) and 10 LR-HPV genotypes (HPV6, 11, 40, 42, 43, 44, 55, 61, 81 and 83); (5) HPV DNA (23 genotypes) Diagnostic Kit (Sansure Biotech Co, Hunan, China) which tests for 18 HR-HPV genotypes (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82) and 5 LR-HPV genotypes (HPV6, 11, 42, 43 and 81).

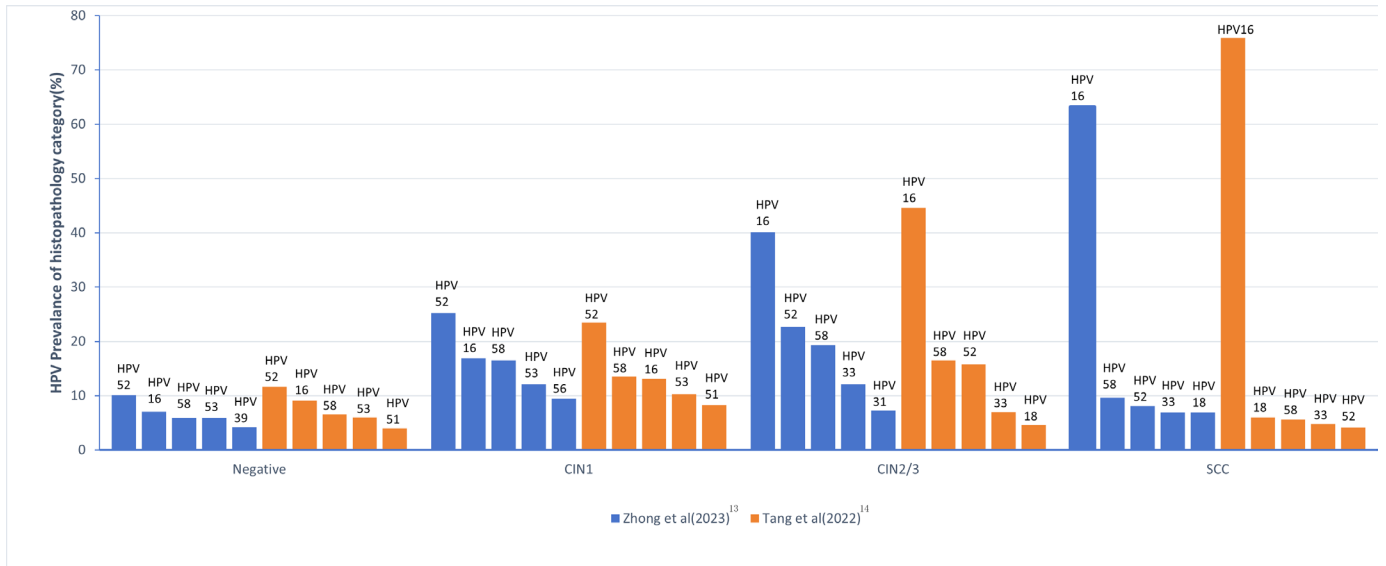
## 2. DISTRIBUTION OF HPV GENOTYPES IN HISTOPATHOLOGICALLY CONFIRMED CERVICAL PRECANCEROUS LESIONS AND CANCERS

HPV-dependent cervical epithelial lesions generally include cervical intraepithelial neoplasia 1 (CIN1), CIN2/3, endocervical adenocarcinoma in situ (AIS) and invasive cervical carcinomas: squamous cell carcinoma (SCC), ADC, adenosquamous carcinoma (ADSC), neuroendocrine carcinoma (NEC), etc. The HR-HPV infection rates have been shown to increase progressively with the severity of cervical squamous lesions, but this pattern is not seen with glandular lesions. In the studies by Zhong *et al* and Tang *et al*, the patients were stratified into three age groups: <30 years, 30–49 years, and 50 years and above. HR-HPV prevalence was highest in the <30 years group in all categories except SCC and ADC.<sup>13 14</sup> As shown in figure 1, HPV52, 58 and 53 are involved primarily in the benign or low-grade cervical squamous lesions, while HPV16 is mainly associated with more severe lesions (CIN2/3 and SCC). HPV18 is significantly more common in glandular lesions than in squamous lesions (figures 1 and 2).<sup>13 14</sup>

### 2.1 Histologically negative findings and CIN1

Histologically negative results provide baseline data of infection status in the normal population. CIN1 is the mildest of HPV-dependent cervical lesions, mostly self-regresses and frequently only requires follow-up. The biological behaviour of CIN1 is the closest to that of negative cases, as compared with other cervical intraepithelial lesions.

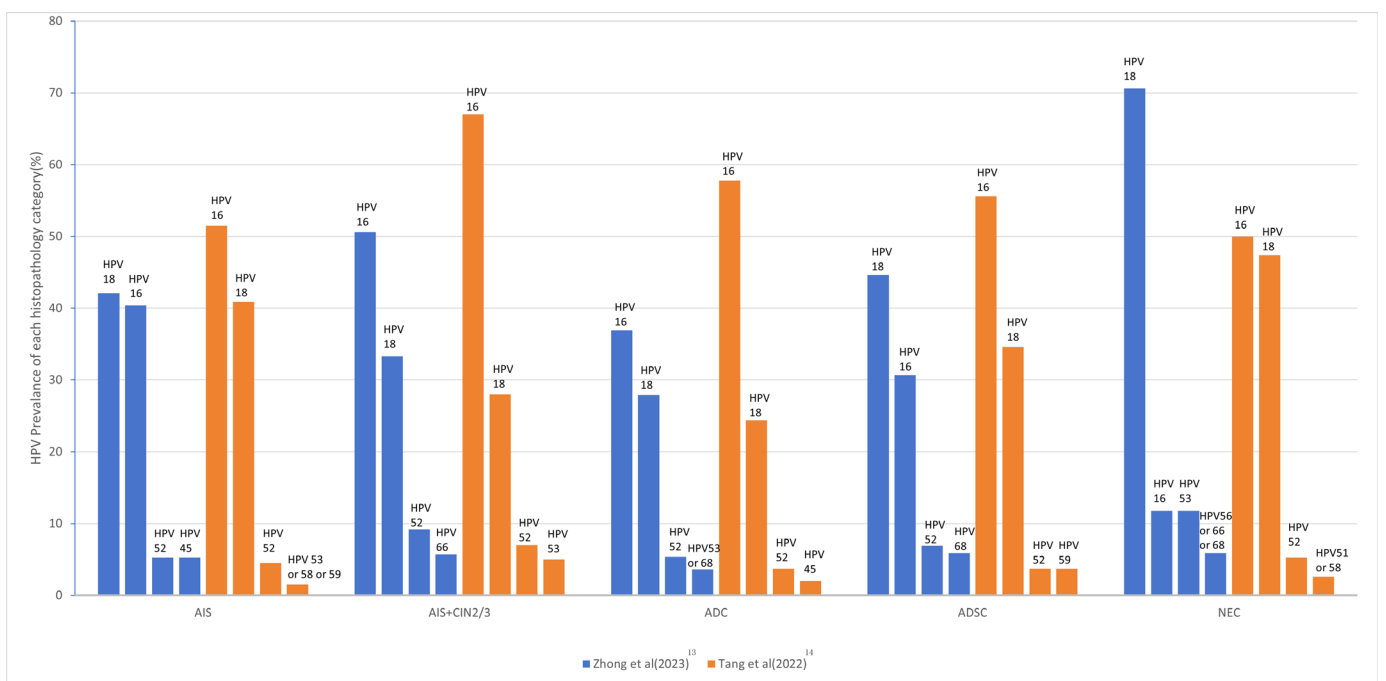
The prevalence of HR-HPV infection in negative cases was 36.2% (Beijing city), 43.9% (Shanghai city) and 44.9% (Chengdu city), respectively, by three independent large-scale retrospective studies.<sup>7 13 14</sup> The relatively higher HR-HPV positivity rate in histologically biopsied negative specimens may be due to selective bias. These women underwent biopsy due to abnormal screening



**Figure 1** HR-HPV genotyping for histologically negative cases and cervical squamous lesions. CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; SCC, squamous cell carcinoma.

results; the study populations are not representative of the general Chinese population.<sup>13 14</sup> By age stratification, the HR-HPV infection rates were 14.8% (<30 years), 11.9% (30–49 years), 11.3% (≥50 years) in Beijing city; 58.2% (<30 years), 40.9% (30–49 years), 43.8% (≥50 years) in Shanghai city; and 55.7% (<30 years), 41.6% (30–49 years), 47.7% (≥50 years) in Chengdu city. HR-HPV prevalence was highest in the <30 years group in all three studies. In these three studies, the top three most prevalent HR-HPV genotypes were HPV52 (10.1–28.5%), HPV58 (5.9–16.3%) and HPV16 (7.1–12.4%), which showed variable

positivity rates. There are very limited data on HR-HPV prevalence for women with histologically biopsied negative findings in Western countries. The data from Europe showed the three most common HR-HPV genotypes were HPV16 (2.3%), HPV18 (0.7%) and HPV31 (0.6%) in normal cytology.<sup>15</sup> Data from the USA demonstrated the three most common HR-HPV genotypes were HPV16 (4.1%), HPV53 (1.1%) and HPV18 (1.0%) in normal cytology.<sup>15</sup> In comparison with biopsy-negative cases, the CIN1 group has a higher HR-HPV infection rate at 88.4% (Shanghai city), 75.7% (Chengdu city), 69.2% (Europe)



**Figure 2** HR-HPV genotyping for histologically cervical glandular lesions. ADC, adenocarcinoma; ADSC, adenosquamous carcinoma; AIS, adenocarcinoma in situ; CIN, cervical intraepithelial neoplasia; HR-HPV, high-risk human papillomavirus; NEC, neuroendocrine carcinoma.



and 79.3% (USA), respectively.<sup>13–15</sup> Similar to the negative group, the CIN1 group also showed decreased trends with increasing age for HR-HPV prevalence: 90.7% (<30 years), 87.9% (30–49 years), 87.4% (≥50 years) in Shanghai city, and 79.0% (<30 years), 74.1% (30–49 years), 76.8% (≥50 years) in Chengdu city.<sup>13 14</sup> The top three genotypes of HR-HPV in the CIN1 groups are HPV52 (25.2% and 23.5%), HPV58 (16.5% and 13.5%) and HPV16 (16.9% and 13.1%) among Chinese cities.<sup>13 14</sup> The similar findings in HPV genotype and age distribution between CIN1 and histologically negative cases support follow-up-only as an optimal clinical management for most CIN1 cases. In Europe and the USA, the HPV distribution in CIN1 is somewhat different, with the top three HPV genotypes being HPV16 (21.6%), HPV31 (10.4%), HPV51 (6.9%) and HPV16 (19.4%), HPV51 (12.5%), HPV56 (10.6%), respectively.<sup>15</sup>

## 2.2 CIN2/3 and SCC

The distribution of HPV genotypes in CIN2/3 and SCC has a close correlation, regardless of the morphology, aetiology and genetics. In a large study of HPV genotyping in Shanghai, China,<sup>13</sup> the HR-HPV positive rates for CIN2/3 and SCC were 92.8% and 93.7%, respectively. When the results were stratified into three age groups, HR-HPV prevalence for CIN2/3 was the highest in the <30 years group (94.3%), followed by the 30–49 years group (92.8%) and ≥50 years group (92.1%). In contrast, the opposite trend was found in SCC with a gradually increasing positive rate at 85.7% (<30 years), 92.1% (30–49 years) and 94.9% (≥50 years). In CIN2/3 lesions, the five most common HPV genotypes were HPV16 (40.1%), HPV52 (22.7%), HPV58 (19.3%), HPV 33 (12.1%) and HPV31 (7.3%). In SCC, the most prevalent genotypes were HPV16 (63.2%), HPV58 (9.6%), HPV52 (8.1%), HPV33 (6.9%) and HPV18 (6.9%). CIN2/3 and SCC had the same top four genotypes with HPV16 as the most prevalent genotype, while HPV16 infection is present much more frequently in SCC than in CIN2/3. These results were similar to the finding of Jiang *et al*'s study from Chengdu City, China.<sup>16</sup>

A study including data from Europe and America illustrated differences between HPV18 distribution.<sup>15</sup> In Europe, the five most common HPV genotypes in CIN2/3 were HPV16 (51.8%), HPV31 (10.0%), HPV33 (8.6%), HPV18 (6.0%) and HPV52 (3.6%). For SCC in the European cohort, the five most common genotypes were HPV16 (57.9%), HPV18 (15.8%), HPV33 (4.4%), HPV31 (4.0%) and HPV45 (2.9%). In the USA, the five most common HPV genotypes seen in CIN2/3 were HPV16 (46.0%), HPV6 (9.9%), HPV18 (9.6%), HPV31 (9.4%) and HPV58 (6.7%), whereas the most common genotypes in SCC were HPV16 (54.8%), HPV18 (21.6%), HPV31 (3.7%), HPV33 (3.5%) and HPV45 (3.3%). The HPV18 distribution shows marked differences in CIN2/3 and SCC among studies of China, Europe and the USA.<sup>13–16</sup>

When CIN2 and CIN3 were separately studied, Tang *et al* demonstrated that CIN2 had a distinctive prevalence

pattern for HPV genotypes 16, 33, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 compared with CIN1 and CIN3, which provided a genotypical basis for the three-tiered classification of squamous intraepithelial lesions.<sup>14</sup>

## 2.3 Endocervical glandular lesions

AIS is the precursor lesion of HPV-dependent ADC of the cervix. AIS often coexists with high-grade squamous intraepithelial lesions (CIN2/3). HPV positivity rates vary across various endocervical glandular lesions: 87.7–95.5% in AIS, 92.0–99.0% in AIS+CIN2/3, 62.0–83.3% in ADC, 76.9–94.1% in ADSC and 82.4–100% in NEC.<sup>4 13 14 16–19</sup> Zhong *et al*<sup>13</sup> demonstrated that HR-HPV positivity rates were significantly lower in glandular lesions (87.7% in AIS and 70.3% in ADC) as compared with squamous epithelial lesions. When the glandular lesions coexisted with squamous lesions (AIS+CIN2/3 and/or ADSC), their positivity rates were elevated (92.0%, 94.1%), similar to the rates for pure squamous lesions. HR-HPV prevalence was highest in the <30 years group in all three categories except ADC.<sup>13 14</sup>

Only seven HPV genotypes were detected in the AIS including HPV 18 (42.1%), followed by HPV16 (40.4%), HPV45 (5.3%), HPV52 (5.3%) and HPV 53, 58, 59 (each 1.8%)<sup>13</sup>; this result was similar to the study of Tang *et al*, where they found that AIS was associated with eight HPV genotypes: HPV16 (51.5%), HPV18 (40.9%), followed by other 6 HPV genotypes including HPV52 (4.5%), HPV35, 45, 53, 58, 59 (each 1.5%).<sup>14</sup> Similarly to the distribution in AIS, ADC displays HPV16 (57.8%) and HPV18 (24.4%) as the two main genotypes, followed by HPV52 (3.7%), HPV45 (2.0%) and HPV58 (1.7%).<sup>14</sup> A study from 38 countries found HPV16, 18 and 45 were detected in 94% (443 of 470 cases) of ADC. HPV18 and HPV45 together were more common in ADC than in SCC (44% vs 14%,  $p < 0.0001$ ).<sup>4</sup>

In terms of concurrent squamous and glandular lesions, the distribution pattern of HR-HPV genotypes in AIS+CIN2/3 shows a concentration of HPV16 (50.6%) and HPV18 (33.3%). In ADSC, HPV16 (30.7%) and HPV18 (44.6%) are the predominant HPV genotypes. These findings from the study of the Chinese population demonstrated that mixed cervical squamous and glandular lesions have similar HPV genotype distribution patterns to that of glandular lesions. The result also illustrated a distinction from squamous lesions, whose patterns were more enriched with HPV16, HPV58 and HPV52, and less so with HPV18 infection.<sup>13 14</sup> In addition, HPV18 was the most common or second common HPV genotype in NEC, similar to AIS, ADC and ADSC (figure 2).<sup>4 13 14 16</sup>

## 3. MULTIPLE HR-HPV INFECTIONS

In contrast to a single HR-HPV infection, the detection of multiple HR-HPV genotypes was not uncommon, with a prevalence from 24.3% to 38.3% in HR-HPV-positive cases, depending on geographical regions.<sup>6–8 13 14 20</sup>

Among coinfection with multiple HR-HPV, dual infection was consistently most common with a prevalence of 18.1% in HR-HPV-positive cases, followed by triple HPV infection of 4.7%, and by four or more HPV types of 1.9%.<sup>6</sup> Other studies from China showed similar results.<sup>20 21</sup> In the context of age, multiple infections occurred more frequently in women younger than 30 years than in older women.<sup>6 13 14</sup>

The prevalence and distribution pattern of multiple HR-HPV infections varied among different cervical diseases, with the clinical significance of multiple HR-HPV infections remaining controversial. Recently, Zhong *et al* analysed multiple infections in different histological groups and found that they occurred most commonly in CIN1 cases (41.3%, 3672 of 8896), while least commonly in ADSC cases (16.8%, 16 of 95).<sup>13</sup> The study also showed that multiple HPV infections occurred more commonly in squamous lesions (38.4%) than in glandular lesions (21.8%). Multiple HPV infections also occurred more frequently in precancerous lesions such as CIN2/3 as compared with SCC (34.3% vs 23.3%). Additionally, Tang *et al* also found that biopsy-negative histopathological cases are the second most common in multiple HR-HPV infections (26.1%), after CIN1 cases.<sup>14</sup> They also observed a decrease in multiple infections with increasing severity of squamous lesions (33.8% in CIN1, 23.6% in CIN2, 18.9% in CIN3, 10.9% in SCC), while such a trend was not evident with cervical glandular lesions (7.9% in AIS, 12.8% in ADC, 12.2% in ADSC, 17.1% in NEC). With respect to HPV distribution, the three most commonly detected HR-HPV genotypes in multiple infections were HPV52, 58 and 16.<sup>8 21–24</sup>

Chaturvedi *et al* have suggested that coinfection with multiple HPV occurs at random,<sup>25</sup> whereas other studies found some HPV genotypes demonstrated preferences when coinfecting with other genotypes.<sup>21 26</sup> For example, HPV16 was more frequently coinfecting with HPV51 and 52 compared with other genotypes,<sup>26</sup> and HPV16/18 were most often coinfecting with HPV31, 52 and 58.<sup>21</sup> To date, multiple HR-HPV infections and an association with high-grade lesions remain debatable. Some studies have observed that multiple infections may synergistically affect the risk of high-grade lesions compared with single infection,<sup>22 25 27 28</sup> while the study by Chaturvedi *et al* showed little evidence for synergistic interactions.<sup>25</sup> To study the clinical significance of concomitant multiple HR-HPV infections in squamous lesions, Tang *et al* explored the association of single and multiple coinfections of HR-HPV in CIN3+ (CIN3 and SCC) lesions in a large cohort (n=24 361).<sup>8</sup> The results demonstrated the risk of CIN3+ detection rate was not increased in multiple (two or three) HR-HPV coinfections, as compared with single HPV infection; conversely, it was decreased in the infections with some combinations of HR-HPV genotypes. These findings were supported by Zhong *et al*,<sup>20</sup> who demonstrate that multiple HR-HPV infections are not associated with cumulatively higher risk of CIN2+ development, suggesting competitive and/or cooperative interactions among HPV genotypes.

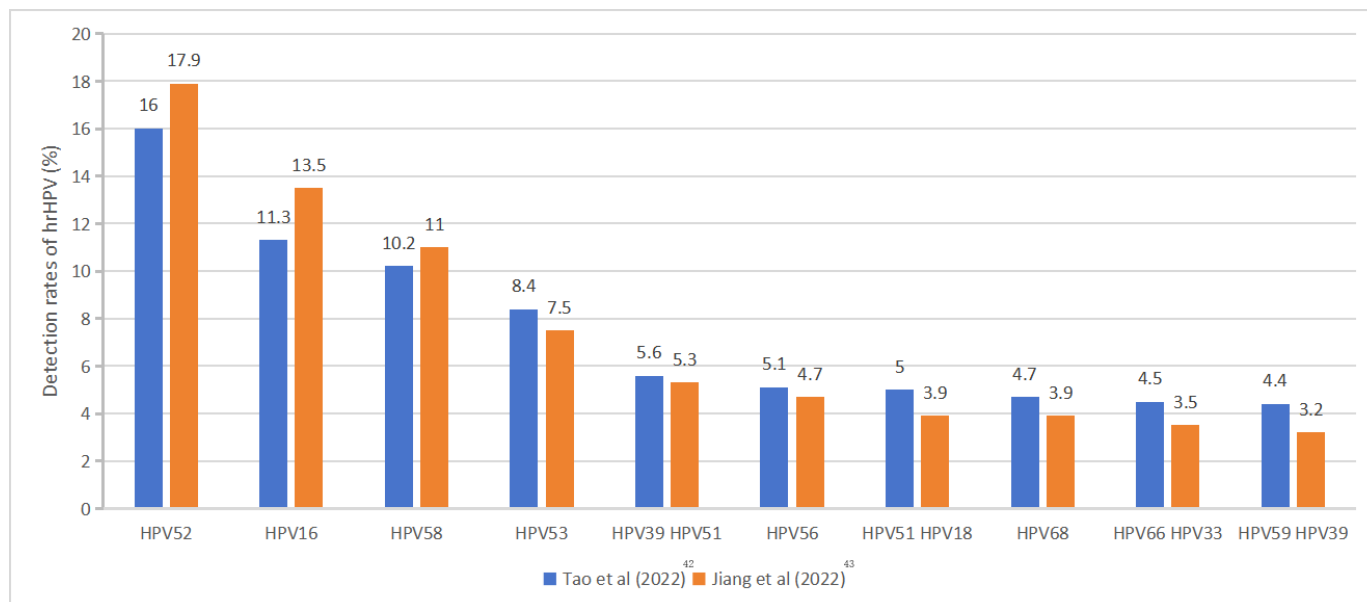
#### 4. HPV GENOTYPES IN CERVICAL CYTOLOGY

The Bethesda System (TBS) for reporting cervical cytology includes the diagnoses of negative for intraepithelial lesion or malignancy (NILM), ASC-US, low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells, cannot exclude HSIL (ASC-H), high-grade squamous intraepithelial lesion (HSIL), SCC and atypical glandular cells (AGC).<sup>29</sup> In 2020, the American Cancer Society recommended that the cervical cancer screening strategy includes primary HPV testing, but that co-testing and cytology alone were alternatives if primary HPV testing was not available.<sup>10</sup> This was in line with the management guidelines of the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP).<sup>30</sup> Overall, a combination of HPV testing, paired with genotyping, and cervical cytology can identify the risk of precancer and further determine the need for colposcopy or treatment.

##### 4.1 Negative for intraepithelial lesion or malignancy

A certain proportion of cytology-negative cases are found to be HR-HPV positive, with a prevalence of 1.9–16.0% in the USA<sup>31–35</sup> and 13.12–17.0% in China.<sup>24 36 37</sup> Management of this group of patients has been controversial. The 2019 ASCCP recommended that women 30 years or older with Pap-negative/HR-HPV-positive results should be followed by either repeat co-testing at 12 months or immediate HPV genotype-specific testing for HPV16 alone or HPV16/18. Colposcopy could be considered for patients who underwent HPV genotyping and were found to be positive for HPV16 or HPV18.<sup>30</sup> The support for genotyping in this setting arose from long-term observational studies in the USA that indicated an elevated risk of high-grade CIN and cervical cancer in HPV16/18-infected women with HPV-positive/cytology-negative results.<sup>35 38</sup> In a large-scale study in the Chinese population,<sup>37</sup> the overall HPV16/18 prevalence was 24.7%, with 17.9% being HPV16 positive, 6.2% being HPV18 positive and 0.6% being positive for both HPV16 and 18 among the 18 423 cytology-negative cases in this cohort. Subsequent histological examination demonstrated that CIN2+ lesions were most commonly diagnosed in HPV16-positive only (15.2%), followed by HPV16 and HPV18 (9.6%), HPV18 only (4.8%), another non-HPV16/18 high-risk genotype (3.0%) and HPV negative (0.8%). The difference between these groups was significant. These findings indicate enhanced risk stratification with HPV16/18 genotype testing in HPV-positive, cytology-negative women.

Although HPV16 positivity carried the highest risk of all genotypes for developing high-grade cervical lesions, other studies also observed relatively high-risk estimates among women positive for HPV18, HPV31 and HPV33, with the 8-year absolute risk developing of CIN3+ being 21.8% for HPV16, 12.8% for HPV18, 11.3% for HPV31 and 12.9% for HPV33, respectively.<sup>35</sup> Given that HPV16, 18 or 45 was detected in 94% of cervical ADC,<sup>39</sup> Han *et al*<sup>40</sup> calculated that the CIN2+ detection rate for HPV16/18/45 (11.5%; 14 of 122 cases) was higher than



**Figure 3** HR-HPV genotyping in women with ASC-US cytology. ASC-US, atypical squamous cells of undetermined significance; HR-HPV, high-risk human papillomavirus.

that for the HPV16/18/45-negative genotypes (3.6%; 7 of 196 cases). Additionally, three cases of stage I cervical ADC were diagnosed in HPV16/18/45-positive and cytology-negative cases. These findings reflected that HPV16/18/45-positive and cytology-negative patients were taken colposcopic procedure and then helped early detection of cervical ADC. In short, it is recommended that women with Pap-negative/HR-HPV-positive results have an HPV genotyping test, and those who are positive for HPV16, 18 or 45 should be considered for colposcopy.

#### 4.2 Atypical squamous cells of undetermined significance

ASC-US represents a screening challenge, due to its indeterminate abnormal cytology results and various underlying aetiologies for atypia, including non-neoplastic conditions, HPV-independent lesions, CIN and cervical cancer. HPV testing is recommended among women with an ASC-US Pap result according to the ASCCP guidelines.<sup>30</sup> Which genotype is more oncogenic in HPV-positive/ASC-US cases has been investigated. Studies have reported an HR-HPV-positive rate of 33.7–66.9% in women with ASC-US cytology, with the most prevalent genotypes being HPV52, 16 and 58 (figure 3).<sup>41–45</sup> The detection rate of single and multiple HPV genotypes in ASC-US/HR-HPV-positive cases was 65.9% vs 34.1% and 77.2% vs 22.8% in two studies, respectively.<sup>42 43</sup> When all cases were stratified into five age groups (<30 years, 30–39 years, 40–49 years, 50–59 years, and 60 years and above), single HPV infection was highest in the 40–49 years group in Tao *et al*'s study,<sup>42</sup> whereas it was highest in the 30–39 years group in the study of Jiang *et al*.<sup>43</sup> Multiple HPV infections were highest in ≥60 years age groups in both studies.<sup>42 43</sup> Tao *et al* observed that both CIN1 and CIN2+ detection rates increased as the number of HR-HPV genotypes increased, suggesting a synergistic effect of multiple HR-HPV genotypes on cervical oncogenesis.<sup>42</sup>

Jiang *et al* found that the CIN2+ diagnostic rate (33.1%) was somewhat higher in a single HPV16 infection than in multiple infections including HPV16 (32.7%).<sup>43</sup> These observations suggest that synergistic or competitive relationships depend on genotype. Some studies discuss the risk stratification for cervical neoplasia not only using extended HR-HPV genotyping, but also combined with viral load testing in women with ASC-US cytology.<sup>41–48</sup> Global studies examining the relationship of various HR-HPV genotypes with the highest risk of CIN2+ have shown the following associations: HPV16/18/31/33/58, 16/33/82/18/31, 16/31/35/45/82, 16/18/33/51/52 and 16/58/18/33/31 in different Chinese populations (table 1),<sup>41–43 45 46</sup> HPV16/31/52/58 in Korean populations,<sup>44</sup> and 16/18/31/33/58 and 16/33/31/18 in various US populations.<sup>47 48</sup> These results have consistently identified HPV16 as the genotype associated with the highest risk of CIN2+, while HPV18 shows variable increased risk. HPV82, which is not included among genotypes detected in the HPV assay approved by the US FDA, was reported as the third or fifth genotype with the highest risk of CIN2+. Moreover, Wang *et al*'s study concluded that high HPV viral loads have a high risk of HSIL+, but a significantly increased viral load was associated only with HPV16-related CIN2+.<sup>42 45</sup>

#### 4.3 Low-grade squamous intraepithelial lesion

ASCCP guidelines recommended direct colposcopy for women aged 25 years and older who have LSIL regardless of HPV status.<sup>30</sup> Some studies stated that extended HR-HPV genotyping was of great use for risk stratification and efficient triage of HR-HPV+/LSIL cytology.<sup>49 50</sup> The prevalence of HR-HPV was high (73.6–84.0%), but there was a small portion of CIN2+ lesions (8.5–10.9%) identified in women who had HR-HPV+/LSIL cytology results on immediate follow-up.<sup>49 51 52</sup> This implies that most

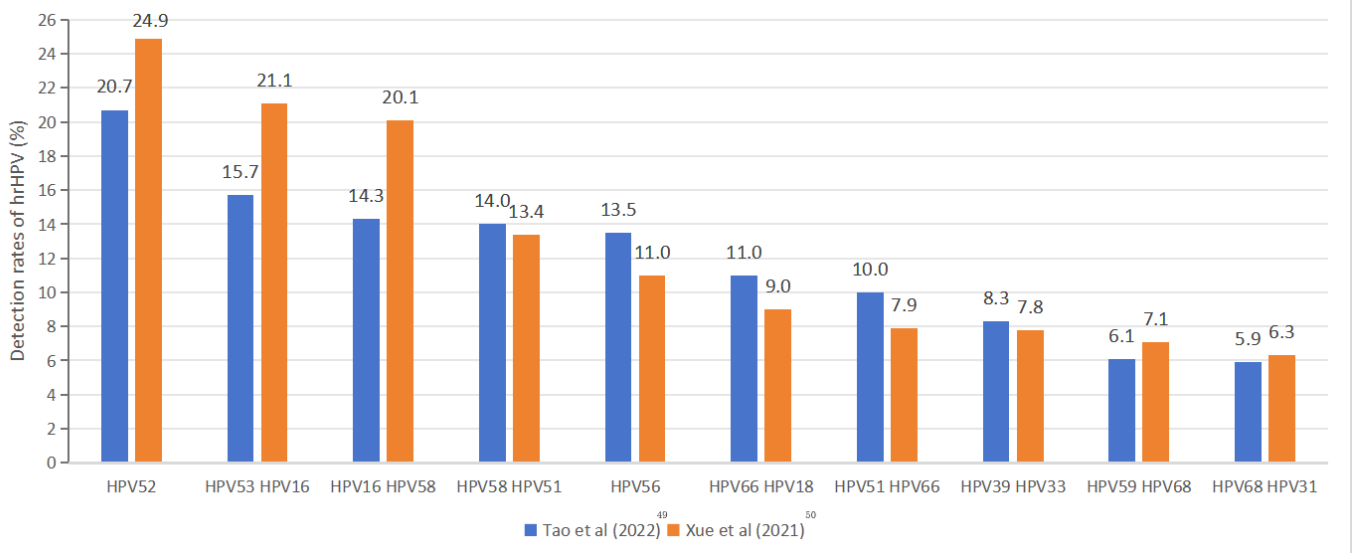
**Table 1** Rates of CIN2+ histopathological diagnoses in women with ASC-US cytology

HR-HPV type	Tao et al <sup>42</sup>		Jiang et al <sup>43</sup>		
	Case no	CIN2+ No Percentage	Case no	CIN2+ No Percentage	Percentage
HPV16	1065	202 19.0	1239	405 32.7	
HPV18	365	30 8.2	350	62 17.7	
HPV26	32	3 9.4	1	0 0	
HPV31	340	34 10.0	180	54 30.0	
HPV33	346	54 15.6	295	76 25.8	
HPV35	211	17 8.1	115	29 25.2	
HPV39	502	16 3.2	283	26 9.2	
HPV45	100	9 9.0	66	16 24.2	
HPV51	453	22 4.9	414	48 11.6	
HPV52	1480	104 7.0	1489	303 20.3	
HPV53	709	33 4.7	504	48 9.5	
HPV56	420	21 5.0	353	39 11.0	
HPV58	951	69 7.3	870	201 23.1	
HPV59	367	17 4.6	234	38 16.2	
HPV66	363	13 3.6	170	27 15.9	
HPV68	408	17 4.2	316	45 14.2	
HPV73	75	3 4.0	42	5 11.9	
HPV82	67	10 14.9	39	12 30.8	

ASC-US, atypical squamous cells of undetermined significance; CIN2+, cervical intraepithelial neoplasia two and above lesions; HPV, human papillomavirus; HR-HPV, high-risk HPV; No, number.

women with LSIL cytology do not require further colposcopic examination. The five most prevalent HR-HPV genotypes from two regions in China were HPV52 (20.7%), HPV53 (15.7%), HPV16 (14.3%), HPV58 (14.0%) and HPV56 (13.5%), and HPV52 (24.9%), HPV16 (21.1%), HPV58 (20.1%), HPV58 (13.4%) and HPV56 (11.0%), HPV66 (11.0%), HPV18 (9.0%), HPV51 (10.0%), HPV66 (7.9%), HPV39 (8.3%), HPV33 (7.8%), HPV59 (6.1%), HPV68 (7.1%), HPV68 (5.9%) and HPV31 (6.3%), respectively (figure 4).<sup>49 50</sup> The top five CIN2+ associated HPV infections showed a similar pattern, with HPV16 (25.2%), HPV82 (17.8%), HPV33 (16.3%), HPV31 (14.6%), HPV26 (13.8%), and HPV16 (44.2%), HPV58 (21.2%), HPV52 (17.7%), HPV18 (13.7%), HPV33 (8.0%), respectively (table 2).<sup>49 50</sup> A study

HPV56 (11.0%), respectively (figure 4).<sup>49 50</sup> The top five CIN2+ associated HPV infections showed a similar pattern, with HPV16 (25.2%), HPV82 (17.8%), HPV33 (16.3%), HPV31 (14.6%), HPV26 (13.8%), and HPV16 (44.2%), HPV58 (21.2%), HPV52 (17.7%), HPV18 (13.7%), HPV33 (8.0%), respectively (table 2).<sup>49 50</sup> A study



**Figure 4** HR-HPV genotyping in women with LSIL cytology. HR-HPV, high-risk human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.



**Table 2** Rates of CIN2+ histopathological diagnoses in women with LSIL cytology

HR-HPV type	Tao <i>et al</i> <sup>49</sup>		Wright <i>et al</i> <sup>52</sup>			
	Case no	CIN2+ No	Percentage	Case no	CIN2+ No	Percentage
HPV16	616	155	25.2	1239	100	44.2
HPV18	208	19	9.1	350	31	13.7
HPV26	29	4	13.8	1	—	—
HPV31	213	31	14.6	180	13	5.8
HPV33	208	34	16.3	295	18	8.0
HPV35	150	10	6.7	115	7	3.1
HPV39	341	16	4.7	283	2	0.9
HPV45	61	5	8.2	66	3	1.3
HPV51	437	21	4.8	414	12	5.3
HPV52	918	72	7.8	1489	40	17.7
HPV53	702	34	4.8	504	—	—
HPV56	585	26	4.4	353	8	3.5
HPV58	650	70	10.8	870	48	21.2
HPV59	275	18	6.5	234	6	2.7
HPV66	420	16	3.8	170	10	4.4
HPV68	257	17	6.6	316	11	4.9
HPV73	58	7	12.1	42	—	—
HPV82	45	8	17.8	39	—	—

CIN2+, cervical intraepithelial neoplasia two and above lesions; HPV, human papillomavirus; HR-HPV, high-risk HPV; LSIL, low-grade squamous intraepithelial lesion; No, number.

by Tao *et al* showed that for the composite group with HPV types HPV16, 26, 82, 31, 18, 33, 58, 35, 52 and 51, the risk of CIN2+ was 11.5% and represented 97.1% of all CIN2+ in biopsied, HR-HPV-positive patients, while other eight genotypes represent a low CIN2+ risk (0.8%) in LSIL cytology cases, which was similar to that of HR-HPV negative (1.5%).<sup>49</sup> The study also reported that multiple HR-HPV infections were identified in 42.8% of HR-HPV-positive cases and were more common in women younger than 30 years. Except for HPV16, 18, 26 and 82, the detection rate of CIN2+ was higher for any (single or multiple) HPV infection than those for a single infection. Interestingly, the CIN2+ detection rate for single HPV genotype infection was 7.2%, significantly increased by coinfections of two HR-HPV genotypes (9.9%) but did not additionally increase by coinfections of  $\geq 3$  HR-HPV genotypes (9.9%).<sup>49</sup> Xue *et al* proposed an HPV16/18/31/33/52/58 genotyping model showed better efficacy in the detection of CIN2+ lesions in HR-HPV-positive LSIL cases than the ASCCP-recommended HR-HPV non-genotyping model.<sup>50</sup> This proposed model demonstrated the potential to reduce the unnecessary colposcopy referral burden in China. The study of Wright *et al* using Onclarity HPV genotyping found that risk associated with HPV35/39/68/45, and 56/59/66 was relatively low and beneath the benchmark threshold risk for immediate colposcopy in ASC-US or LSIL cytology.<sup>52</sup> When restricted to women aged  $\geq 50$

years, Zhang *et al* found that the detection rate of CIN2/3 lesions in HR-HPV-negative cases was very low (0.5%). Close follow-up may be appropriate for these women.<sup>51</sup> This supported the recent ASCCP guideline of optional deferral of colposcopy for postmenopausal women with LSIL cytology and negative HPV results.<sup>30</sup>

#### 4.4 Atypical squamous cells, cannot exclude HSIL

ASC-H is a heterogeneous diagnostic category with cytological changes suggestive of HSIL, accounting for 5–10% of atypical squamous cells in TBS for reporting cervical cytology. Kaiser Permanente Northern California Medical Care Plan data indicated that ASC-H confers a higher risk of CIN3+ over time than ASC-US or LSIL,<sup>53 54</sup> and the high rate of HPV detection in women with ASC-H makes reflex HPV testing unsuitable.<sup>55</sup> Both the 2012 and 2019 ASCCP guidelines recommended that for women with ASC-H cytology, colposcopy should be performed regardless of HPV result. They also noted that reflex HPV testing is not recommended in this setting.<sup>30 56</sup> Many studies have demonstrated that HPV-positive rates in women with ASC-H Pap test results were 50–60% and women with ASC-H Pap with negative HPV tests would have a very low risk of high-grade cervical lesions.<sup>57–59</sup> In the USA, many laboratories provide reflex HPV testing for ASC-H Pap.<sup>60 61</sup> HPV testing results can provide useful risk stratification information in women with ASC-H Pap tests.<sup>62</sup>



HPV genotyping data are rare for women with ASC-H Pap test results. Recently, a study from China reported HPV genotyping results from 1414 women with ASC-H Pap tests.<sup>63</sup> In this study, ASC-H had a higher HR-HPV infection rate of 84.4% and a higher risk of CIN2+ (46% for CIN2/3, 8.9% for cervical carcinoma) than a diagnosis of LSIL. The prevalence of multiple HR-HPV coinfections was 24.4% in total and was highest in women <30 years (30.4%). The CIN2+ detection rates by single HR-HPV genotype infection, two-genotype coinfections and three or more genotype coinfections were 55.1%, 67.8% and 62.5%, respectively, with a trend similar to that of LSIL. The five most common HR-HPV genotypes were HPV16 (34.9%), HPV52 (22.2%), HPV58 (13.4%), HPV33 (8.5%) and HPV31 (6.8%). Among these, HPV16 had the highest CIN2+ detection rate of 70.5%, followed by HPV31 with a rate of 70.0%. It is worth noting that 16.4% (21 of 128) of women with HR-HPV-negative ASC-H cytology results had CIN2+ lesions, and 3.9% (5 of 128) had invasive carcinoma. These data were different from that of a previous study, in which 1.6% (14 of 885) of women with HR-HPV-negative ASC-H results showed CIN2+ lesions but no cervical carcinoma after an average follow-up period of 29 months.<sup>58</sup> In all, there seems no sufficient evidence for a triage role of HR-HPV genotyping in the ASC-H group.

#### 4.5 High-grade squamous intraepithelial lesion

The HR-HPV infection rate of HSIL is generally high (over 90–95%). The five most common HPV genotypes are HPV16 (95.24%), HPV18 (47.62%), HPV52 (28.57%), HPV58 (16.67%), HPV33 (9.52%), and HPV16 (48.3%), HPV58 (19.9%), HPV52 (18%), HPV33 (11.5%), HPV53 (5.9%) from two studies in China.<sup>24 36</sup> Patients 25 years old or older with HSIL cytology and non-HPV16/18-positive results are generally referred to coloscopy, but in the context of HSIL cytology with HPV16 positivity, an expedited excisional treatment is preferred.<sup>30</sup>

#### 4.6 Atypical glandular cells

AGC may be the most challenging diagnosis in cytopathology, due to difficulty in sampling and morphological interpretation. A significant proportion of glandular lesions are HPV independent, which makes cervical cancer screening strategies that are based on HPV testing less effective. The 2019 ASCCP guidelines recommended colposcopy with endocervical sampling for AGC cytology (except in the case of atypical endometrial cells) and additional endometrial sampling for women aged ≥35 years or <35 years with clinical indications suggesting a high risk of an endometrial neoplasm such as obesity, unexplained vaginal bleeding and chronic anovulation.<sup>30</sup> Triage by reflex HPV testing is not recommended by the guideline. Many studies indicated that the HPV positivity rate is about 20–40% for women with AGC Pap, and the women with AGC Pap and negative HPV testing results would have a very low risk of high-grade squamous or glandular lesions.<sup>64–68</sup> A study from Sweden documented

a high HPV positivity rate of 72.9% (647 of 888 cases). Furthermore, HPV16 and HPV18 have been the most prevalent genotypes identified in AGC cases.<sup>69</sup> Yilmaz *et al* reported the five most common types in their cohort were HPV16 (19.8%), HPV18 (15.6%), HPV31 (9.5%), HPV45 (5.8%) and HPV52 (4.4%), while Tang *et al* identified HPV16 (16.7%), HPV18 (9.5%), HPV52 (7.1%), HPV58 (6.6%) and HPV53 (3.6%) in their study population.<sup>66 69</sup> A study from Italy revealed HPV58 and HPV31 to be the most common types after HPV16 and HPV18.<sup>70</sup> In the study of AGC by Pradhan *et al*, the most severe lesion was CIN2/3 (5.6%), followed by endometrial carcinoma (5.5%) and endocervical lesions (1.3% for AIS, 0.6% for ADC) after histological follow-up within 1 year.<sup>67</sup> With respect to HPV results suggesting the risk of high-grade lesions in AGC cytology, it is mostly agreed that a positive HR-HPV result significantly increased the risk of developing CIN2+ (CIN2/3, AIS, ADC), and HPV16 or HPV18 further increased the risk.<sup>64–66 71</sup> Whether HR-HPV is positive or not does not affect the evaluation of endometrial adenocarcinoma risk, whereas older age (≥50 years) increases the risk of developing endometrial carcinoma with a cytological diagnosis of AGC.<sup>64</sup> Some studies assess non-HPV16/18 type-specific risk for high-grade cervical lesions among women with AGC. Yilmaz *et al* revealed that HPV31 and 33 had a higher risk of CIN3+, with HPV45 infection harbouring an increased risk of cervical carcinoma.<sup>69</sup> Schiffman *et al* found that the presence of HPV45 alone, or in combination with AGC cytology, indicated an elevated risk of AIS and cervical carcinoma.<sup>72</sup> Tang *et al* reported the increasing risk of CIN2+ for the composite group of HPV16/18/58/52/53-positive cases among HR-HPV-positive AGC cases.<sup>66</sup> It then follows that the combination of cytology, HPV genotyping and patient age may aid in the risk assessment and appropriate management of these patients.

#### 5. SUMMARY

CIN2/3 and SCC had the same top four genotypes with HPV16 as the most prevalent genotype, while HPV16 infection is present much more frequently in SCC than in CIN2/3. ADC displays HPV16 and HPV18 as the two main genotypes. In terms of concurrent squamous and glandular lesions, the distribution of HR-HPV genotypes shows a similar pattern to glandular neoplasias. Significant differences have been noted in the oncogenicity of HR-HPV genotypes. HPV16, HPV18 and HPV45 are by far the most common and most oncogenic genotypes in the USA and European countries, while HPV18 and HPV45 are less frequent in China and Asian countries, especially in squamous lesions. Multiple HR-HPV infections are very common, but multiple HR-HPV infections are not associated with cumulatively higher risk of high-grade cervical squamous lesion development. Extensive HPV genotyping allows for risk stratification and optimises clinical management. When HPV results are positive for genotypes other than HPV16 or HPV18,

additional information is important for determining the need for colposcopy for women with mildly abnormal Pap tests. Clinical guidelines based on additional genotypes (so-called extended genotyping) are in development.

Most extensive HPV genotyping findings in this review are from the studies in China and other Asian countries because HPV genotyping data are very limited in the USA and European countries.

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