Journal of
Molecular Pathology

MDPI

Article

Comparison of the Hybrid Capture II Method with a PCR-Based Screening Method Using a Carboxyfluorescein-Labeled Primer for Detecting Human Papillomavirus in Cervicovaginal Liquid-Based Cytology

Yusuke Saiki [1,2], Yuka Gion [1,3,*], Asami Nishikori [1], Yoshiaki Norimatsu [4] and Yasuharu Sato [1,3]

1 Divison of Pathophysiology, Okayama University Graduate School of Health Sciences, Okayama 700-8558, Japan; scsaiki@outlook.jp (Y.S.); pp3b7xoa@s.okayama-u.ac.jp (A.N.); satou-y@okayama-u.ac.jp (Y.S.)
2 Department of Clinical Laboratory, Saijo Central Hospital, Saijo 793-0027, Japan
3 Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan
4 Department of Medical Technology, Ehime Prefectural University of Health Sciences, Iyo-gun 791-2101, Japan; ynorimatsu@epu.ac.jp
* Correspondence: gion@okayama-u.ac.jp; Tel.: +81-86-235-7150; Fax: +81-86-235-7156

Received: 8 July 2020; Accepted: 7 September 2020; Published: 10 September 2020

Abstract: Objective: Human papillomaviruses (HPVs) are DNA viruses, of which over 120 types have been identified. The main screening methods for HPV-DNA include the hybrid capture II (HC-II) and polymerase chain reaction (PCR) assays. Liquid-based cytology (LBC) is a high-quality technique developed to improve the diagnostic reliability of traditional Papanicolaou tests (Pap tests). However, relatively few studies have compared the efficacy of PCR and HC-II assays using cervicovaginal LBC specimens. In this study, we conducted a comparative analysis with results derived from the HC-II assay to assess whether a PCR-based assay using a novel carboxyfluorescein (FAM)-labeled primer could be applied to cervicovaginal LBC specimens. Methods and Results: We analyzed 59 specimens diagnosed as atypical squamous cells of undetermined significance (ASCUS) by Pap tests. After extracting DNA from cervicovaginal LBC specimens, we performed PCR using a FAM-labeled consensus primer, and then conducted fragment analysis to confirm the results. The value of the kappa statistic measuring the agreement between the PCR and HC-II results was 0.8557, or "almost perfect agreement." Conclusion: Our novel HPV-PCR assay can be successfully applied to cervicovaginal LBC specimens for the detection of HPV subtypes.

Keywords: human papillomavirus; polymerase chain reaction; hybrid capture II assay; liquid-based cytology; cervical cancer

1. Introduction

Human papillomaviruses (HPVs) are DNA viruses, of which over 120 types have been identified. HPVs are categorized into non-oncogenic, low-risk types (types 6 and 11), and oncogenic, high-risk types (types 16, 18, 31, 33, 35, 52, 58, and others) [1–3]. Condyloma acuminata (genital warts) are caused by low-risk HPV subtypes (6 and 11) and have a low risk of progression to malignancy [4,5]. In contrast, high-risk HPVs are known to induce cervical intraepithelial neoplasia, which can develop

into invasive cancer [1–3]. The rate of progression to malignancy is approximately 10-20%. In particular, HPV 16 and 18 have been reported to be detected in approximately 70% of cervical cancer cases [6–9].

Approximately 300,000 deaths from cervical cancer are reported globally every year, making it the second most lethal cancer in women [10,11]. Regular Papanicolaou tests (Pap tests) are vital to detect precancerous lesions and prevent their progression into cervical cancer. However, despite the numerous studies that have demonstrated that Pap testing has drastically reduced the incidence of cervical cancer, the diagnostic sensitivity of Pap tests for detecting cervical intraepithelial neoplasia above grade 2 (CIN2+) is relatively poor and is estimated to be only 53-65% [12–15]. However, HPV-DNA testing has been reported to have a diagnostic sensitivity of greater than 90% for CIN2+ lesions [15–17]. Accordingly, it has been anticipated that early diagnosis of cervical cancer can be greatly improved by using both Pap tests and HPV-DNA tests. However, combining these two tests has been shown to improve detection sensitivity by only 5%, compared with the sensitivity of the HPV-DNA test alone [18]. Therefore, European countries have begun to adopt the practice of performing HPV-DNA testing first, followed by Pap tests only if the DNA test result is positive.

The hybrid capture II (HC-II; QIAGEN, Valencia, CA, USA) technique involves the hybridization of the HPV-DNA specimen with an RNA probe cocktail and capturing the resulting DNA-RNA hybrid onto the surface of a microplate well coated with antibodies specific to the DNA-RNA hybrids, which yields a chemiluminescent readout. As this method does not involve the amplification of HPV-DNA and instead combines the HC-II method with chemiluminescence, it has a high sensitivity for the detection of HPV-DNA. This method has also been proven with respect to its clinical specificity and sensitivity, and is therefore widely used in Japan as well [19]. HC-II is performed for atypical squamous cells of undetermined significance (ASCUS) cases in Pap tests. In recent years, PCR-based genotyping analysis has also been adopted as a screening method to detect HPV-DNA [20,21]. Although PCR amplification greatly increases the signal sensitivity for HPV-DNA, the excessive amplification can lead to unnecessary medical tests and treatments. Therefore, there is such a problem in clinical application of highly sensitive HPV-PCR. Although comparisons of the HPV-DNA detection rates between PCR and HC-II assays have shown that PCR has a greater detection rate, most studies have reported that PCR results are largely in agreement with HC-II results [22–24]. On the other hand, there have been reports that the results from PCR and HC-II are not consistent [25]. In addition, many countries have begun to recognize the usefulness of liquid-based cytology (LBC) specimen collection, which was developed to improve the homogeneity of Pap test specimens and the standardization of results [26,27]. However, only a few studies have assessed whether the results derived from PCR assays on cervicovaginal LBC specimens agree with HC-II results. Furthermore, to our knowledge, no studies have compared the HPV-DNA detection rates of PCR assays involving a carboxyfluorescein-labeled primer with those of HC-II assays.

Since it is widely known that HPV affects cell morphology, HPV infection in ASCUS was examined using HC-II and PCR. In this study, we describe a procedure for HPV-DNA detection using PCR with a novel carboxyfluorescein-labeled primer. We conducted fragment analysis using a high-sensitivity capillary sequencer to confirm the PCR results. To assess whether this PCR-based technique for HPV screening can be applied to the detection of HPV-DNA from specimens collected through cervicovaginal LBC, we also performed a comparative analysis with results from HC-II assays.

2. Methods and Materials

2.1. Samples

The study included a total of 59 patients diagnosed with indeterminate ASCUS by Pap tests using cervicovaginal LBC specimens from Saijo Central Hospital (Saijo, Japan) and who underwent the HC-II test.

Pap tests were screened by one cytotechnologist in routine medical examination. A total of 59 LBC specimens were collected between July 2018 and January 2019. LBC samples for Pap tests were usually collected with a brush, or cotton swabs were used for pregnant women.

All samples underwent HC-II testing by an outsourcing company (BML, Tokyo, Japan). The cervicovaginal samples were collected with a Cervex-Brush® (Rovers Medical Devices B.V., Lekstraat, The Netherlands), then fixed in a BD SurePath™ collection vial (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for Pap staining. The specimens used for HC-II included one residual clinical sample from a Pap test collected through cervicovaginal LBC and 58 new samples taken with swabs (10 cases) or brushes (48 cases). Resampling of HC-II analysis was performed within about 1 month of diagnosing ASCUS.

Comprehensive informed consent was obtained for all subjects in the form of opt-out in the Saijo Central Hospital, and the study was approved by the ethics committee of the Saijo Central Hospital (approval date: 23 February 2018). All procedures were performed in accordance with the Helsinki Declaration

2.2. HPV Detection by PCR

2.2.1. DNA Extraction

DNA was extracted from 2 mL of preservation liquid from the BD SurePath™ collection vial using a QIAamp DNA Micro Kit (QIAGEN, Valencia, CA, USA). The DNA concentration was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.2.2. PCR

We performed PCR using the TaKaRa Taq™ (Takara Bio, Tokyo, Japan). The primers were designed to target the E6 and E7 genes of HPV. We used the forward primer: 5′-TGTCAAAAACCGTTGTGTCC-3′, to detect high-risk HPV types (types 16, 18, 31, 33, 35, 52b, and 58), and the forward primer (5′-TGCTAATTCGGTGCTACCTG-3′) for detecting low-risk HPV types (types 6 and 11). A carboxyfluorescein (FAM)-labeled consensus primer (5′-GAGCTGTCGCTTAATTGCTC-3′) was inserted into both forward primers. PCR was performed using the following conditions: denaturation at 94 °C for 30 s, annealing at 55 °C for 1 min, and elongation at 72 °C for 30 s, for a total of 40 cycles.

2.2.3. Fragment Analysis

We performed capillary electrophoresis on PCR products using the ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems, Foster, CA, USA) and analyzed the data using the ABI PRISM® GeneMapper Software Version 3.0 (Applied Biosystems). We defined a specimen to be HPV-positive when a peak corresponding to a band size of 228 to 268 bp was detected (Figure 1).

2.3. Statistical Analysis

We used the kappa statistic categorization system described by Landis et al. [28] as a reference and defined values of 0.81-1.00 to be "almost perfect agreement," 0.61-0.80 to be "substantial agreement," 0.41-0.60 to be "moderate agreement," 0.21-0.40 to be "fair agreement," 0.00-0.20 to be "slight agreement," and <0.00 to be "poor agreement".

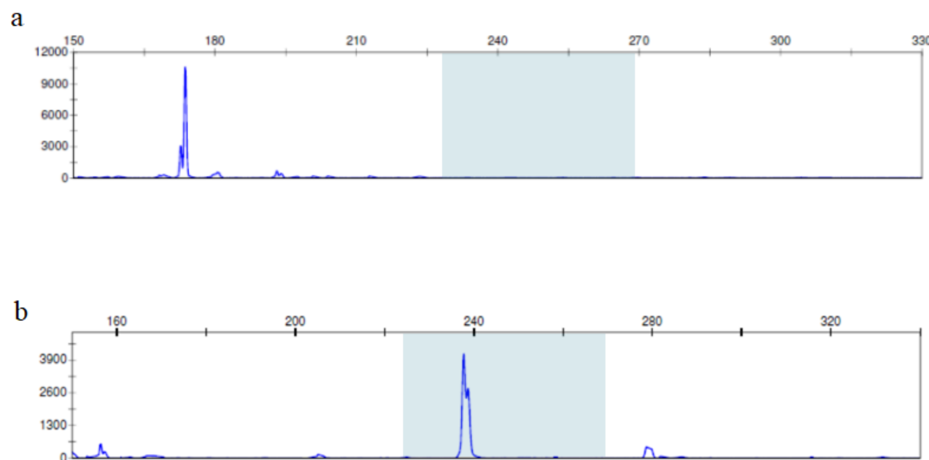

Figure 1. Criteria for fragment analysis. (**a**) Negative case, no peak at the expected fragment size of 228–268 bp; the 170-bp peak is a nonspecific peak for the human genome (**b**) Positive case, a peak is detected from the PCR product, representing a fragment of 228–268 bp in size.

3. Results

The mean age of the patients was 44.4 years (Table 1). High-risk HPV subtypes were detected in 7 of 59 patients (11.9%) using the PCR assay and in 9 of 59 patients (15.3%) using the HC-II assay. Low-risk HPV-DNA was detected in 3.4% of the cases (2/59) by PCR assays (the HC-II assay only detects high-risk subtypes), and both assays produced negative results for the remaining 50 patients.

Table 1. Patient ages and human papillomavirus (HPV) detection results produced by hybrid capture II (HC-I)I assay and PCR.

Age (Years)	n (%)	PCR+ (High-Risk)	PCR+ (Low-Risk)	HC-II+
20–29	4 (6.8%)	2 (28.6%)	0	3 (33.3%)
30–39	22 (37.3%)	3 (42.9%)	1 (50.0%)	4 (44.4%)
40–49	15 (25.4%)	2 (28.6%)	0	2 (22.2%)
50–59	11 (18.6%)	0	0	0
60–69	5 (8.5%)	0	1 (50.0%)	0
70–79	1 (1.7%)	0	0	0
80–89	1 (1.7%)	0	0	0
Total	59	7	2	9
Range	22–80	24–47	30–69	24–47
Mean	44.4	35.1	49.5	34.6
Median	45	35	49.5	35

n: number of cases.

When the results from both assays were compared by age groups, the detection rates for high-risk HPV in specimens collected from patients in their twenties were 28.6% (2/7) and 33.3% (3/9) using PCR and HC-II, respectively; 42.9% (3/7) and 44.4% (4/9) for specimens collected from patients in their thirties; and 28.6% (2/7) and 22.2% (2/9) for patients in their forties. We did not detect any high-risk HPV subtypes in the specimens collected from patients in their fifties to eighties (Table 1).

Overall, the detection results of high-risk HPV subtypes produced by PCR and HC-II assays were in agreement for 96.6% cases (57/59); the agreement for the positive results was 77.8% (7/9); and the agreement for the negative results was 100% (50/50) (Table 2). The value of the kappa statistic measuring the agreement between the PCR and HC-II results was 0.8557 or "almost perfect agreement."

Table 2. Comparing high-risk HPV-DNA detection results from PCR and HC-II assays.

		HC-II		Total
		+	**−**	
PCR	+	7 (78%)	0 (0%)	7
	−	2 (22%)	50 (100%)	52
Total		9	50	59

4. Discussion

In this study, we determined that the detection of high-risk HPV-DNA using PCR and HC-II assays yielded results that were in complete agreement in 96.6% of the cases. In addition, the kappa statistic of 0.8557 confirmed the "almost perfect agreement" of the two assays. The value of the kappa statistic can be applied to the evaluation of data categorized into two or more categories, but it is difficult to use it for interpretation of comparisons with other studies with different proportions of subjects in a category. Previous studies have reported that the agreement between PCR and HC-II results for HPV-DNA detection is over 80% [23,24,29]. The results from our study exhibited a higher agreement than did those reported by Tsiodras et al. ($\hat{e}$ = 0.691) [22] and Kulmala et al. ($\hat{e}$ = 0.669) [23] in Europe, as well as Peyton et al. ($\hat{e}$ = 0.63) [24] in Central America. The HPV subtypes targeted by the HC-II assay in the current study include the same 13 high-risk types assessed by Kulmala et al. [23] and Peyton et al. [24], namely: HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 [30]. However, the HC-II assay performed by Tsiodras et al. [22] included five low-risk HPV types (HPVs 6, 11, 42, 43, and 44). Using the HC-II assay, HPV-DNA was detected in 33.7% of cases (509/1511) by Kulmala et al. [23], 12.5% (26/208) by Peyton et al. [24], and 20.4% (260/1270) by Tsiodras et al. [22]. Peyton et al. [24] also performed PCR on 20 types of HPV in addition to the 13 high-risk types cited above and produced positive detection results for these subtypes as well. The HPV subtypes detected by PCR in the studies by Tsiodras et al. and Kulmala et al. also covered a variety of HPVs. While we targeted seven types of high-risk HPV in our PCR assay, Tsiodras et al. [22] targeted 22 types, and Kulmala et al. [23] targeted 12 types (Table 3). PCR assays detected HPV-DNA in 36.6% of cases (553/1511) in the study by Kulmala et al. [23], 24.5% (51/208) by Peyton et al. [24], and 31.3% (397/1270) by Tsiodras et al. [22]. We hypothesize that the different subtypes targeted in each study influenced the rates of agreement between the results produced by PCR and HC-II (Tsiodras et al. $\hat{e}$ = 0.691, Kulmala et al. $\hat{e}$ = 0.669, Peyton et al. $\hat{e}$ = 0.63). In addition, the sample size of our HPV-DNA positive cases was small. The agreement of 78% for the positive results is likely unstable (Table 2). Therefore, the true agreement for the positive results may be lower than 78%. The validation guidelines for candidate HPV assays proposed by Meijer et al. [31] suggest that the sensitivity of the candidate test for ≥CIN2 should be at least 90% of the sensitivity of the HC-II; at the same time, the specificity of the candidate test should be at least 98% of the specificity of HC-II. In this study, the sensitivity of PCR was 78% of that of HC-II, and the specificity was 100% of that of HC-II. Therefore, our HPV-PCR may not be a candidate test for ≥CIN2. However, the value of the kappa statistic measuring the agreement between the PCR and HC-II results was 0.8557 or "almost perfect agreement." Thus, it is necessary to conduct further analysis for ≥CIN2.

Table 3. HPV subtypes detected in published studies using PCR-based tests.

Author	Year	n	Details of HPV Subtypes
Tsiodras S et al. [22]	2010	1270	6, 11, 13, 16, 18, 30, 31, 32, 33, 35, 39, 40, 43, 45, 51, 52, 54, 55, 56, 58, 59, 66
Kulmala SM et al. [23]	2004	1511	16, 18, 31, 33, 35, 39, 45, 51, 52, 54, 56, 58
Peyton CL et al. [24]	1998	208	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 (+20 subtypes: detail omission)

In our study, we found two cases where the PCR produced a negative result, whereas the HC-II assay yielded a positive result. This was probably because the HC-II test detects HPV subtypes (39, 45, 51, 56, 59, 68) that were not targets of our PCR assay. In Japan, HPV subtypes are detected in the order of 52, 16, 58, 56, 51, and 18 in CIN1 patients and healthy persons [32]. However, although we targeted fewer HPV types than the other PCR-based studies performed previously, our data showed a 96.6% (57/59) agreement score between the PCR and HC-II results. In our study, all of the specimens that were found to be HPV-positive by PCR (types 16, 18, 31, 33, 35, 52, and 58) were also found to be positive by HC-II testing. The PCR test developed by Tsiodras et al. [22] was able to detect four additional types of HPV compared to the HC-II assay, and an additional seven types could only be analyzed by PCR. However, Kulmala et al. [23] reported only one type of HPV that could only be analyzed by PCR. Thus, the high rate of agreement in our results may be attributed to the fact that we have not included the HPV types that can be detected only by PCR in our study. In addition, we surmise that the presence of HPV types that are particularly common in Japan (types 52 and 58) may have partially influenced the high rate of agreement in our results [33,34].

It should be noted that each of the reports that have been discussed thus far had different parameters and clinical characteristics with respect to sample selection. Tsiodras et al. [22] analyzed 1270 specimens from women who had Pap test results ranging from normal to cancerous. Kulmala et al. [23] analyzed 1511 specimens from gynecological patients (including patients with sexually transmitted diseases (STDs)) and those who had reported in for screening. Peyton et al. [24] selected 208 specimens that, aside from 10 with small irregularities, were found to be normal. Of the specimens selected by Tsiodras et al., 81% (1029/1270) were assessed as normal by Pap tests. Among the cases evaluated as normal, HPV-DNA was detected in 17.98% of the samples (185/1029) by PCR assays, while HC-II tests yielded positive results for 10.20% of cases (105/1029) [22]. Given that these studies largely targeted specimens that did not show any abnormalities in Pap tests, we surmise that their results may have included temporary HPV infections; thus, the viral load and sensitivity of methods for HPV-DNA may have contributed to the lower agreement scores. We suggest that when comparing agreement scores for different screening methods for HPV-DNA, it is important to take into consideration which HPV types have been targeted and any regional variances, as well as accounting for different clinical characteristics.

In our study, we showed 100% (50/50) agreement between the negative results detected by both PCR and HC-II. Generally, PCR has a higher sensitivity and can detect as little as 10 copies of HPV-DNA in a single sample [35,36]. HC-II was developed based on the principles of Southern blotting and is the most widely used assay in clinical settings, with a DNA detection rate of 290 copies in one sample [37]. As such, the high sensitivity of PCR might increase the number of false positives. However, we did not obtain any cases where a specimen was given a positive result for a high-risk HPV type by PCR and not by HC-II. For specimens where both procedures gave a negative result, we can thus rule out infection with HPV types detectable by the two methods (types 16, 18, 31, 33, 35, 52, and 58). It is, however, possible that the negative results included cases where there was a non-zero number of DNA copies representing HPV types not targeted by our PCR test, but which also fell below the 290-copy detection threshold for HC-II.

With respect to analytical sensitivity, it is debatable whether the presence of extremely low levels of HPV-DNA has any clinical relevance. Of the patients who tested positive for high-risk HPV types, 70% were diagnosed with ASCUS according to Pap tests and were infected with other HPVs other

than 16 or 18, putting them at a lower risk for CIN2 [38]. Furthermore, many cases of HPV infection in female patients under the age of thirty are temporary, with only a few patients developing any symptoms; even those who have progressed to CIN2 may recover completely in two to three years [39]. For such patients, it is possible that a high-sensitivity screening method may result in unnecessary interventions. In many cases of cervical cancer, immunological surveillance mechanisms are engaged and result in HPV elimination. The activation of cellular immune defenses against the E6 and E7 proteins is particularly important for such immunological surveillance and is influenced by factors such as the individual's immune function and the environment [40]. In this context, the detection of minute levels of high-risk HPV types (including type 16) may also better inform patient monitoring and provide grounds for intervention. At the other end of the age spectrum, in patients over fifty years of age with ASCUS Pap test diagnoses, it has been reported that the probability of false negatives for CIN2+ from the HC-II assay increases with age [41], suggesting that it may be more effective to screen older patients through PCR rather than HC-II.

In our research, we detected two incidences of low-risk HPV-DNA (3.4%) using a PCR-based test. This is comparable to the results of Beyazit et al. [42], where 2 out of 33 cases (6.1%) were detected in a study targeting HPV types 6 and 11 in ASCUS specimens. Similarly, a report by Castle et al. [4] observed that the rates of singular infection by HPV types 6, 11, and 42 was less than 2% (in specimens for which Pap test results ranged from negative to squamous intraepithelial lesions and atypical glandular cells). The HPV subtypes 6 and 11 were not found to be correlated with precancerous cervical lesions within three years of detection [4], although low-risk HPV types have been identified in over 90% of cases reporting genital growths, specifically condyloma acuminata [5]. To follow up on the two cases where low-risk HPV was detected, we conducted colposcopy examinations of the patients. One of the examinations revealed the presence of a white external pudendal protrusion. Although we did not conduct further pathological examination of this tissue, we suspected it to be a genital growth resulting from HPV type 6 or 11. Genital growths are one of the most common forms of STDs and are characterized by cellular changes such as koilocytosis, which often accompany HPV infection. Thus, cases where koilocytosis was present were automatically determined to be abnormal in Pap tests. In such cases, HPV screening that includes only selected subtypes of high-risk HPV cannot determine whether the cellular abnormality originates from low-risk HPV subtypes or from other high-risk HPV subtypes that are not targeted by the screening test. PCR-based assays, using primers containing HPV types 6 and 11, are an effective method for differentiating between low-risk HPV types and other high-risk HPV types that are not targeted by the conventional screening tests. They also allow for more accurate data to be acquired, which can inform patient monitoring. We suggest that PCR-based assays targeting HPV types 6 and 11 can provide important information in cases where genital growths are apparent.

5. Conclusions

The detection results from our HPV-PCR using a FAM-labeled primer demonstrated almost perfect agreement with the results generated from HC-II assays, suggesting that our HPV-PCR can be successfully applied to specimens collected through cervicovaginal LBC. Our novel HPV-PCR also detected DNA from both high-risk and low-risk HPV types. While the HC-II method requires no special apparatus or equipment, our HPV-PCR is equally convenient, and can be easily performed in a clinical laboratory.

Author Contributions: Conceptualization, Y.G. and Y.S. (Yasuharu Sato); Methodology, Y.G.; Validation, Y.G. and A.N.; Writing—Original Draft Preparation, Y.S. (Yusuke Saiki); Writing—Review & Editing, Y.G. and Y.S. (Yasuharu Sato); Supervision, Y.S. (Yasuharu Sato) and Y.N.; Project Administration, Y.G. and Y.S. (Yasuharu Sato). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors report no conflicts of interest.

References

1. Muñoz, N.; Bosch, F.X.; De Sanjose, S.; Herrero, R.; Castellsagué, X.; Shah, K.V.; Snijders, P.J.; Meijer, C.J.L.M. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med.* **2003**, *348*, 518–527. [CrossRef] [PubMed]
2. Bosch, F.X.; Manos, M.M.; Muñoz, N.; Sherman, M.; Jansen, A.M.; Peto, J.; Schiffman, M.H.; Moreno, V.; Kurman, R.; Shan, K.V.; et al. Prevalence of human papillomavirus in cervical cancer: A worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J. Natl. Cancer Inst.* **1995**, *87*, 796–802. [CrossRef]
3. Khan, M.J.; Castle, P.E.; Lorincz, A.T.; Wacholder, S.; Sherman, M.; Scott, D.R.; Rush, B.B.; Glass, A.G.; Schiffman, M. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *J. Natl. Cancer Inst.* **2005**, *97*, 1072–1079. [CrossRef]
4. Castle, P.E.; Hunt, W.C.; Langsfeld, E.; Wheeler, C.M. Three-year risk of cervical precancer and cancer after the detection of low-risk human papillomavirus genotypes targeted by a commercial test. *Obs. Gynecol.* **2014**, *123*, 49–56. [CrossRef]
5. Giuliano, A.R.; Tortolero-Luna, G.; Ferrer, E.; Burchell, A.N.; de Sanjose, S.; Kjaer, S.K.; Muñoz, N.; Schiffman, M.; Bosch, F.X. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. *Vaccine* **2008**, *26*, K17–K28. [CrossRef] [PubMed]
6. Wright, T.C.J.; Stoler, M.H.; Sharma, A.; Zhang, G.; Behrens, C.; Wright, T.L. Evaluation of HPV-16 and HPV-18 genotyping for the triage of women with high-risk HPV+ cytology-negative results. *Am. J. Clin. Pathol.* **2011**, *136*, 578–586. [CrossRef] [PubMed]
7. Thomsen, L.T.; Frederiksen, K.; Munk, C.; Junge, J.; Iftner, T.; Kjaer, S.K. 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. *Int. J. Cancer* **2015**, *137*, 193–203. [CrossRef] [PubMed]
8. Clifford, G.M.; Smith, J.S.; Plummer, M.; Muñoz, N.; Franceschi, S. Human papillomavirus types in invasive cervical cancer worldwide: A meta-analysis. *Br. J. Cancer* **2003**, *88*, 63–73. [CrossRef] [PubMed]
9. Schiffman, M.; Castle, P.E.; Jeronimo, J.; Rodriguez, A.C.; Wacholder, S. Human papillomavirus and cervical cancer. *Lancet* **2007**, *370*, 890–907. [CrossRef]
10. Lowy, D.R.; Solomon, D.; Hildesheim, A.; Schiller, J.T.; Schiffman, M. Human papillomavirus infection and the primary and secondary prevention of cervical cancer. *Cancer* **2008**, *113*, 1980–1993. [CrossRef]
11. Ferlay, J.; Shin, H.R.; Bray, F.; Forman, D.; Mathers, C.; Parkin, D.M. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int. J. Cancer* **2010**, *127*, 2893–2917. [CrossRef] [PubMed]
12. Mayrand, M.H.; Duarte-Franco, E.; Rodrigues, I.; Walter, S.D.; Hanley, J.; Ferenczy, A.; Ratnam, S.; Coutlée, F.; Franco, E. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. *N. Engl. J. Med.* **2007**, *357*, 1579–1588. [CrossRef] [PubMed]
13. Cuzick, J.; Clavel, C.; Petry, K.U.; Meijer, C.J.; Hoyer, H.; Ratnam, S.; Szarewski, A.; Birembaut, P.; Kulasingam, S.; Sasieni, P.; et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int. J. Cancer* **2006**, *119*, 1095–1101. [CrossRef] [PubMed]
14. Wright, T.C., Jr.; Schiffman, M.; Solomon, D.; Cox, J.T.; Garcia, F.; Goldie, S.; Hatch, K.; Noller, K.L.; Roach, N.; Runowicz, C.; et al. Interim guidance for the use of human papillomavirus DNA testing as an adjunct to cervical cytology for screening. *Obs. Gynecol.* **2004**, *103*, 304–309. [CrossRef] [PubMed]
15. Basu, P.; Mittal, S.; Bhadra Vale, D.; Chami Kharaji, Y. Secondary prevention of cervical cancer. *Best. Pract. Res. Clin. Obs. Gynaecol.* **2018**, *47*, 73–85. [CrossRef]
16. Castle, P.E.; Stoler, M.H.; Wright, T.C., Jr.; Sharma, A.; Wright, T.L.; Behrens, C.M. 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*, 880–890. [CrossRef]
17. Stoler, M.H.; Wright, T.C., Jr.; Sharma, A.; Apple, R.; Gutekunst, K.; Wright, T.L. High-risk human papillomavirus testing in women with ASC-US cytology: Results from the ATHENA HPV study. *Am. J. Clin. Pathol.* **2011**, *135*, 468–475. [CrossRef]

18. Barroeta, J.E.; Adhikari-Guragain, D.; Grotkowski, C.E. Cervical cancer screening in the era of HPV vaccination: A review of shifting paradigms in cytopathology. *Diagn. Cytopathol.* **2017**, *45*, 903–914. [CrossRef]
19. Clavel, C.; Masure, M.; Levert, M.; Putaud, I.; Mangeonjean, C.; Lorenzato, M.; Nazeyrollas, P.; Gabriel, R.; Quereux, C.; Birembaut, P. Human papillomavirus detection by the hybrid capture II assay: A reliable test to select women with normal cervical smears at risk for developing cervical lesions. *Diagn. Mol. Pathol.* **2000**, *9*, 145–150. [CrossRef]
20. Fujinaga, Y.; Shimada, M.; Okazawa, K.; Fukushima, M.; Kato, I.; Fujinaga, K. Simultaneous detection and typing of genital human papillomavirus DNA using the polymerase chain reaction. *J. Gen. Virol.* **1991**, *72*, 1039–1044. [CrossRef]
21. Sasagawa, T.; Minemoto, Y.; Basha, W.; Yamazaki, H.; Nakamura, M.; Yoshimoto, H.; Sakaike, J.; Inoue, M. A new PCR-based assay amplifies the E6-E7 genes of most mucosal human papillomaviruses (HPV). *Virus Res.* **2000**, *67*, 127–139. [CrossRef]
22. Tsiodras, S.; Georgoulakis, J.; Chranioti, A.; Voulgaris, Z.; Psyrri, A.; Tsivilika, A.; Panayiotides, J.; Karakitsos, P. Hybrid capture vs. PCR screening of cervical human papilloma virus infections. Cytological and histological associations in 1270 women. *BMC Cancer* **2010**, *10*, 53. [CrossRef] [PubMed]
23. Kulmala, S.M.; Syrjänen, S.; Shabalova, I.; Petrovichev, N.; Kozachenko, V.; Podistov, J.; Ivanchenko, O.; Zakharenko, S.; Nerovjna, R.; Kljukina, L.; et al. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. *J. Clin. Microbiol.* **2004**, *42*, 2470–2475. [CrossRef] [PubMed]
24. Peyton, C.L.; Schiffman, M.; Lörincz, A.T.; Hunt, W.C.; Mielzynska, I.; Bratti, C.; Eaton, S.; Hildesheim, A.; Morera, L.A.; Rodriguez, A.C.; et al. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. *J. Clin. Microbiol.* **1998**, *36*, 3248–3254. [CrossRef]
25. Saini, R.; Shen, T.H.; Othman, N.H.; Santhanam, J.; Othman, N.; Tang, T.H. Evaluation of polymerase chain reaction (PCR) method and hybrid capture II (HCII) assay for the detection of human papillomavirus in cervical scrapings. *Med. J. Malays.* **2007**, *62*, 206–209.
26. Kirschner, B.; Simonsen, K.; Junge, J. Comparison of conventional Papanicolaou smear and SurePath liquid-based cytology in the Copenhagen population screening programme for cervical cancer. *Cytopathology* **2006**, *17*, 187–194. [CrossRef]
27. Roberts, J.M.; Thurloe, J.K. Comparative sensitivities of ThinPrep and Papanicolaou smear for adenocarcinoma in situ (AIS) and combined AIS/high-grade squamous intraepithelial lesion (HSIL): Comparison with HSIL. *Cancer* **2007**, *111*, 482–486. [CrossRef]
28. Landis, J.R.; Koch, G.G. The measurement of observer agreement for categorical data. *Biometrics* **1977**, *33*, 159–174. [CrossRef]
29. Yamazaki, H.; Sasagawa, T.; Basha, W.; Segawa, T.; Inoue, M. Hybrid capture-II and LCR-E7 PCR assays for HPV typing in cervical cytologic samples. *Int. J. Cancer* **2001**, *94*, 222–227. [CrossRef]
30. de Freitas, A.C.; Gurgel, A.P.; Chagas, B.S.; Coimbra, E.C.; do Amaral, C.M. Susceptibility to cervical cancer: An overview. *Gynecol. Oncol.* **2012**, *126*, 304–311. [CrossRef]
31. Meijer, C.J.; Berkhof, J.; Castle, P.E.; Hesselink, A.T.; Franco, E.L.; Ronco, G.; Arbyn, M.; Bosch, F.X.; Cuzick, J.; Dillner, J.; et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. *Int. J. Cancer* **2009**, *124*, 516–520. [CrossRef]
32. Inoue, M.; Sakaguchi, J.; Sasagawa, T.; Tango, M. The evaluation of human papillomavirus DNA testing in primary screening for cervical lesions in a large Japanese population. *Int. J. Gynecol. Cancer* **2006**, *16*, 1007–1013. [CrossRef]
33. Serrano, B.; Brotons, M.; Bosch, F.X.; Bruni, L. Epidemiology and burden of HPV-related disease. *Best Pract. Res. Clin. Obs. Gynaecol.* **2018**, *47*, 14–26. [CrossRef] [PubMed]
34. Hwang, H.S.; Park, M.; Lee, S.Y.; Kwon, K.H.; Pang, M.G. Distribution and prevalence of human papillomavirus genotypes in routine pap smear of 2,470 korean women determined by DNA chip. *Cancer Epidemiol. Biomark. Prev.* **2004**, *13*, 2153–2156.
35. An, H.J.; Cho, N.H.; Lee, S.Y.; Kim, I.H.; Lee, C.; Kim, S.J.; Mun, M.S.; Kim, S.H.; Jeong, J.K. Correlation of cervical carcinoma and precancerous lesions with human papillomavirus (HPV) genotypes detected with the HPV DNA chip microarray method. *Cancer* **2003**, *97*, 1672–1680. [CrossRef] [PubMed]

36. Nishiwaki, M.; Yamamoto, T.; Tone, S.; Murai, T.; Ohkawara, T.; Matsunami, T.; Koizumi, M.; Takagi, Y.; Yamaguchi, J.; Kondo, N.; et al. Genotyping of human papillomaviruses by a novel one-step typing method with multiplex PCR and clinical applications. *J. Clin. Microbiol.* **2008**, *46*, 1161–1168. [CrossRef]
37. Cox, J.T.; Lorincz, A.T.; Schiffman, M.H.; Sherman, M.E.; Cullen, A.; Kurman, R.J. Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. *Am. J. Obs. Gynecol.* **1995**, *172*, 946–954. [CrossRef]
38. Wong, A.K.; Chan, R.C.; Nichols, W.S.; Bose, S. Invader human papillomavirus (HPV) type 16 and 18 assays as adjuncts to HPV screening of cervical papanicolaou smears with atypical squamous cells of undetermined significance. *Cancer* **2009**, *115*, 823–832. [CrossRef] [PubMed]
39. Safaeian, M.; Solomon, D.; Wacholder, S.; Schiffman, M.; Castle, P. Risk of precancer and follow-up management strategies for women with human papillomavirus-negative atypical squamous cells of undetermined significance. *Obs. Gynecol.* **2007**, *109*, 1325–1331. [CrossRef]
40. Stern, P.L. Immune control of human papillomavirus (HPV) associated anogenital disease and potential for vaccination. *J. Clin. Virol.* **2005**, *32*, S72–S81. [CrossRef]
41. Won, K.H.; Lee, J.Y.; Cho, H.Y.; Suh, D.H.; No, J.H.; Kim, Y.B. Impact of age on the false negative rate of human papillomavirus DNA test in patients with atypical squamous cells of undetermined significance. *Obs. Gynecol. Sci.* **2015**, *58*, 117–123. [CrossRef] [PubMed]
42. Beyazit, F.; Sılan, F.; Gencer, M.; Aydin, B.; Paksoy, B.; Unsal, M.A.; Ozdemir, O. The prevelance of human papillomavirus (HPV) genotypes detected by PCR in women with normal and abnormal cervico-vaginal cytology. *Ginekol. Pol.* **2018**, *89*, 62–67. [CrossRef] [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).