Histopathology of Cervical HPV Lesions

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Only after fully understanding the pathogenic mechanisms of HPV lesions and their interaction with different cofactors such as the microbiota will it be possible to define the most effective strategy for patients. The Pathologist and the HPV test allows identifying women with “high risk” to be included in personalized protocols and targeted follow-up in cynical practice.

Keywords: cervical intraepithelial neoplasia ; cervical cancer ; adenocarcinoma ; squamous carcinoma

1. Epidemiology of HPV

Papillomaviruses are widely distributed in mammals and are species-specific [1]. Human papillomavirus (HPV) cannot be cultivated in tissue cultures or in common experimental animals [1]. They are members of the Papillomaviridae family, have no coating, measure from 50 to 55 nm in diameter, and have an icosahedral capsid of 72 capsomers [2]. Of the approximately 200 genotypes of HPV, subdivided into 14 species, about 40 can infect the epithelial cells (skin or mucous membranes) of the anogenital regions and other areas [3]. Differentiation into types is made based on the characteristics of the L1 protein [4]. HPV was the first virus to be recognized as responsible for cervical cancer (CC). According to the degree of association with invasive tumors, HPV genotypes have been subdivided into: high oncogenic risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), related with an increased risk of developing CC [5]; low oncogenic risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89) associated with no disease most commonly or with benign epithelial lesions (such as anogenital and oropharyngeal warts) [6]; and, finally, HPV with an “undetermined risk” (3, 7, 10, 27, 28, 29, 30, 32, 34, 55, 57, 62, 67, 69, 71, 74, 77, 83, 84, 85, 86, 87, 90, 91) include those whose oncogenicity has not yet been fully defined [7]. It has been established that about 99.8% of CC have a high-risk HPV DeoxyriboNucleic Acid (DNA) sequence, particularly HPV 16 and 18, found in about 70% of invasive carcinomas [8]. The prevalence of infection is very high (70% of sexually active female patients over 25 years old), and most infections tend to regress spontaneously, with or without manifestations of dysplasia; only in some cases can HPV infection become persistent [9]. Data from the scientific literature show that in female patients over 30 years old, persistent high-risk HPV infections play a critical role in predicting the risk of developing CC [8][9][10]. The risk of developing in histopathologic high-grade cervical intraepithelial lesion HSIL (CIN2 and CIN3) or invasive CC is estimated to be much higher in women with persistent high-risk HPV infection, being 11 times higher in the 30–44 age group, 35-fold higher between 45 and 54 years, and 49-fold in those over 50 years of age [11]. The ability of HPV viruses, especially those at high risk, to integrate into infected cells, and to orchestrate a gene expression program that allows the transcription of oncogenic proteins (E6, E7), promotes carcinogenicity [12]. Cervical intraepithelial lesions (CIN) can regress spontaneously, or progress to invasive neoplasia in different percentages depending on their severity. More specifically, histopathologic low-grade cervical intraepithelial lesion LSIL (CIN1) tends to regress spontaneously, particularly in young patients. Ostor and coworkers [13] report that CIN1 subsides spontaneously in 60% of cases, persists in 30%, and can progress in 10% of cases. On the other hand, CIN2 regresses spontaneously in 40%, persists in another 40% of cases, and can progress in 20% of cases; finally, CIN3 can regress in 33% of cases and progress in more than 12% of cases.

2. Screening and Histopathology of Cervical HPV Lesions

The natural history of CC is typically characterized by the progression, over the years, of non-invasive HPV-related precancerous lesions to invasive carcinoma [7][8][9][10][11][12][13]. Cytology (Pap-Test) and human papillomavirus detection (HPV-DNA) are two screening tests whose purpose is to detect CC or precancerous changes at an early stage. Before the development of the HPV-DNA test, the Pap-Test alone was performed every 3 years in women after the onset of sexual activity or in any case from 25 years of age [14]. Today, according to guidelines from different countries, HPV-DNA tests are used for women over 30 or over 25, and a Pap-Test is only done if it gives a positive result [15][16][17][18][19][20]. In fact, globally the recommended method of primary CC screening is the HPV-DNA test, independently of resource settings [21], due to its sensitivity compared to the Pap-Test, even in the presence of a lower specificity especially for the identification

of CIN2 and CIN3 lesions. HPV testing is recommended by the World Health Organization [14] and other guidelines [15][22] even with respect to the Pap-Test which is now considered a secondary test [14]. In fact, the Pap-Test even if it has allowed to tangibly reduce the incidence of invasive carcinoma, however, presenting a variability between different operators, it can lead to the diagnosis of false negatives as well as cases of invasive CC are also reported in the literature in women regularly investigated with the Pap-Test [23]. In view of the crucial role of persistent infection of hr-HPV, the focus has shifted to the use of HPV-DNA testing as a screening test [24] so that access to treatment is increasingly targeted and timely. According to the ASCO guidelines, if the HPV-DNA test results positive, genotyping for HPV 16/18 (with or without HPV 45) and/or Pap-Test are also indicated [21]. In the event of a positive or abnormal result, the HPV-DNA test procedure involves colposcopy and related biopsy [21]. Conversely, in discordant results between the HPV test and cytological examination, it is recommended to repeat the HPV-DNA test one year later, then repeat the test at 12–24 months in case of negativity or colposcopy in women who tested positive [21]. Finally, in cases of CIN2 histological diagnosis, patients should be offered a surgical solution followed by targeted follow-up over time [21]. According to data from the randomized study published by Ronco et al. [25], HPV-research-based screening is more effective than the Pap-Test in preventing CC in women aged 25–60, because it allows an earlier identification of high-grade persistent lesions. In fact, the execution of the HPV-DNA test is useful in stratifying the population according to the degree of risk: a negative test indicates a low risk of developing CC, and so in these controls can be made at longer intervals. Although with differences between the various settings, the HPV-DNA test should be started from the age of 30 in the general female population, regular screening being done with the HPV test validated every 5–10 years, versus 25 years of age in women living with HIV, who should be screened more frequently, every 3–5 years. CC is the fourth most common malignancy among women worldwide, accounting for approximately 7% of all female cancers [26][27]. As reported in the literature, most of the diagnoses of CC can be associated with the presence of HPV infection and, in some studies, these associations can reach levels comparable to almost all cases [28]. Among the high-risk HPV genotypes, variant 16 has the highest affinity for neoplastic progression with over 50% of cases, followed by variant 18 which occurs in 20% of cases; this association tends to vanish in the remaining high-risk genotype up to 5% and even less [29]. Conversely, there does not appear to be a significant difference between the HPV status and the histotype of the carcinomas except for squamous-cell carcinoma (SCC) which is unlikely to be HPV-negative [30], as well as in mixed adeno-squamous form where the HPV positivity may reach up to 86% [31] of cases and also in the vast majority of Adenocarcinoma in situ (AIS) [30]. Vice versa, the prevalence of HPV among adenocarcinoma (AD) types can vary and, according to the International Endocervical Adenocarcinoma Criteria and Classification, ADs are divided in two categories: HPV-associated (HPVA) and not HPV-associated (NHPVA), with well-defined characteristics due to histology HPVA shows more mitotic activity or apoptotic figures than NHPVA. If focal or equivocal HPVA features are visible at ×200, a tumor can be classified as a “limited HPVA” and provisionally diagnosed as NHPVA AD [32]. To this histotype belong different variants such as mucinous (that can show aspects of HPVA and NHPVA), gastric (prevalent NHPVA type), endometrioid and serous carcinomas (extraordinarily rare). NHPVA comprehends histological variants [32] such as gastric, clear cell, serous, endometrioid, or mesonephric carcinomas that notoriously tend to be HPV negative [33] compared to histotypes presenting glandular/villo-glandular/intestinal aspects that appear to have a higher percentage of HPV positivity.

Principal Biomarkers for Cervical Cancer

The study of viral and cellular biomarkers that could be useful for identification of specific stages of cervical intraepithelial lesions related to hrHPV infection is closely linked to the biology of HPV and the different stages from infection through intraepithelial lesion, and, if this is not properly treated, to invasive CC. After hrHPV infection, the infected cervical epithelium begins to proliferate, leading to the transformation into CIN1, 2, and 3 [34][35][36][37]. Progression from a transient to a transformation HPV infection is characterized by a sharp increase in HPV mRNA E6/E7 and protein expression [34][37]. With this in mind, several authors have assessed how the detection of mRNA transcripts belonging to E6/E7 proteins could be helpful for identifying cervical precancers [36][37][38][39][40][41][42]. From these works, researchers can deduce a good sensitivity and specificity of these tests; there are two platforms used for the study of this biomarker (PreTect. Proofer and APTIMA GenProbe). The p16INK4a protein is a cyclin-dependent kinase inhibitor that plays a key role in cell cycle regulation and is upregulated when E7 is overexpressed as in HPV infections, representing an ideal biomarker to define the nature of cervical lesions [43][44], especially if combined in a single test together with Ki-67 [45][46] which is generally used in immunohistochemistry to evaluate the cell proliferation index [47]. Double-stained cytology p16/Ki-67 (approved by FDA on 3 October 2020 [48]) is a qualitative immunocytochemical assay intended for the simultaneous detection of proteins P16INK4a (clone E6H4) and Ki-67 (clone 274-11AC3V1) in cervical specimens of women aged 25–65 years with positive HPV test (high oncogenic risk); it is also indicated for patients aged between 30 and 65 who have to postpone colposcopy or have other risk factors regardless of the result of the HPV test [48]. It is highly expressed in almost 100% of cases of CIN2, CIN3, squamous CC, but is rarely found in benign forms; it is highly expressed in 100% of AIS cases. Several studies have highlighted the ability of the p16 test to identify the neoplastic transformation of cervical cells infected with human papillomavirus, being able to predict with greater precision the underlying cervical intraepithelial

neoplasia of grade 3 or worse [49][50]. Specifically, the ATHENA study, on a group of 7727 patients, showed that p16/Ki-67 dual-stained cytology was significantly ($p < 0.0001$) more sensitive than Pap-Test (74.9% vs. 51.9%) for the triage of HPV-positive women and that specificity was comparable between the two methods [50]. In studies directed by Bergeron and Petry [46][51], as well as by Wright and coworkers, the authors described a p16/Ki-67 dual-stained cytology, that either alone or combined with HPV16/18 genotyping, represents a promising approach as a sensitive and efficient triage for colposcopy of HPV-positive women when primary HPV screening is utilized [50]. These studies led the way in using p16/ki-67 as a reliable tool for risk stratification of HPV-positive women with cervical lesions, reporting elevated sensitivity values similar to those of the HPV test [51][52][53][54][55]. Furthermore, other authors have shown that p16/ki-67 had a higher specificity than the HPV test [45][53][54][55]. These data reinforce the diagnostic value of p16/Ki-67, which represents a useful tool in avoiding further diagnostic investigations such as unnecessary colposcopies given its ability to more precisely identify female patients at increased CIN2 risk. In the field of research into serum biomarkers for the early detection of CC, the activation of Macrophage-Colony Stimulating Factor (M-CSF) and Vascular Endothelial Growth Factor (VEGF) is likely involved in the pathogenesis and spread of CC. In particular, M-CSF is overexpressed in the CC lines compared to CIN and the blocking of the M-CSF receptor determines both a growth arrest and an increase in intratumoral apoptosis [56][57]. In various papers, Lawicki and coworkers [38][58], using the immunosorbent assay (ELAISA), evaluated the plasma levels of M-CSF as compared to commonly accepted tumor markers, such as CA 125 and SCC-Ag in CC patients before surgery and in healthy subjects. The M-CSF plasma level was significantly higher, as also CA 125 and SCC-Ag, in CC patients. The diagnostic sensitivity of M-CSF was higher than stem cell factor, CA 125, and SCC-Ag (25%, 30%, 40%, respectively). The diagnostic specificity was high and equal for all tested cytokines and CA 125 (92%). Positive and negative predictive values were higher for all tested parameters but highest for M-CSF (83% and 69.7%, respectively) [38]. More recent studies [59][60][61][62] confirmed that cytokine members of hematopoietic growth factors may have a diagnostic potential in CC. Zajkowska evaluated serological markers against CA 125 and SCC-Ag in 100 CC patients with chemiluminescent microparticle immunoassay and showed statistically significant M-CSF values from all parameters tested in the CC cohort compared to the control groups [59]. Similar results have also been described by Lubowicka et al. who investigated M-CSF, matrix metalloproteinase-2, and its inhibitor beyond CA 125 and SCC-Ag in 89 CC patients and in 50 healthy women (aged 22–61 years) reporting for M-CSF the highest specificity (86%) in the CC group [60]. Moreover, the association of these different markers increased in specificity, as observed in the combination between matrix metalloproteinase-2 and CA 125 in different CC stages [60]. While median levels of M-CSF and VEGF, as well as CA 125 and SCC-Ag are shown to be significantly different in women with CC, this relationship does not seem to be specific to SCC alone; in fact, the plasma levels of M-CSF and VEGF are also higher in AD than in the control group and, moreover, no significant differences were observed between SCC and AD [61]. Sidorkiewicz reported 81% sensitivity and 74% specificity for VEGF in the SCC group and 86% and 76% in the AD group [61]. Although the results of these studies are encouraging and suggest a diagnostic utility and a possible clinical applicability of serum biomarkers in patients with CC, to date they are not yet sufficiently studied, and more confirmations would be useful for their wider use than p16/Ki67 testing and mRNA detection.

References

1. Brianti, P.; De Flammineis, E.; Mercuri, S.R. Review of HPV-related diseases and cancers. New Microbiol. 2017, 40, 80–85.
2. Soheili, M.; Keyvani, H.; Soheili, M.; Nasseri, S. Human papilloma virus: A review study of epidemiology, carcinogenesis, diagnostic methods, and treatment of all HPV-related cancers. Med. J. Islamic Repub. Iran 2021, 35, 65.
3. Schneider, A.; Koutsky, L.A. Natural history and epidemiological features of genital HPV infection. IARC Sci. Publ. 1992, 119, 25–52.
4. McBride, A.A. Oncogenic human papillomaviruses. Philos. Trans. R. Soc. B Biol. Sci. 2017, 372, 20160273.
5. Salambanga, C.; Zohoncon, T.M.; Traore, I.M.A.; Ouedraogo, R.A.; Djigma, W.F.; Ouedraog, C.; Simpore, J. High prevalence of high-risk human papillomavirus (HPV) infection among sexually active women in Ouagadougou. Med. Sante Trop. 2019, 29, 302–305.
6. Nielsen, A.; Iftner, T.; Norgaard, M.; Munk, C.; Junge, J.; Kjaer, S.K. The importance of low-risk HPV infection for the risk of abnormal cervical cytology/histology in more than 40,000 Danish women. Sex. Transm. Infect. 2012, 88, 627–632.
7. Morandell, D.; Rostek, U.; Bouvard, V.; Campo-Fernandez, B.; Fiedler, M.; Jansen-Durr, P.; Zwerschke, W. Human papillomavirus type 45 E7 is a transforming protein inducing retinoblastoma protein degradation and anchorage-independent cell cycle progression. Virology 2008, 379, 20–29.

8. de Sanjose, S.; Brotons, M.; Pavon, M.A. The natural history of human papillomavirus infection. Best Pract. Res. Clin. Obstet. Gynaecol. 2018, 47, 2–13.
9. Bosch, F.X.; de Sanjose, S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J. Natl. Cancer Inst. Monogr. 2003, 2003, 3–13.
10. Munoz, N.; Bosch, F.X.; de Sanjose, S.; Herrero, R.; Castellsague, X.; Shah, K.V.; Snijders, P.J.; Meijer, C.J. International Agency for Research on Cancer Multicenter Cervical Cancer Study, G. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Engl. J. Med. 2003, 348, 518–527.
11. Chen, H.C.; Schiffman, M.; Lin, C.Y.; Pan, M.H.; You, S.L.; Chuang, L.C.; Hsieh, C.Y.; Liaw, K.L.; Hsing, A.W.; Chen, C.J.; et al. Persistence of type-specific human papillomavirus infection and increased long-term risk of cervical cancer. J. Natl. Cancer Inst. 2011, 103, 1387–1396.
12. Mbuya, W.; Held, K.; McHaro, R.D.; Haule, A.; Mhizde, J.; Mnkai, J.; Mahenge, A.; Mwakatima, M.; Sembo, M.; Mwalongo, W.; et al. Depletion of Human Papilloma Virus E6- and E7-Oncoprotein-Specific T-Cell Responses in Women Living With HIV. Front. Immunol. 2021, 12, 742861.
13. Ostor, A.G. Natural history of cervical intraepithelial neoplasia: A critical review. Int. J. Gynecol. Pathol. 1993, 12, 186–192.
14. WHO. New Recommendations for Screening and Treatment to Prevent Cervical Cancer. 2021. Available online: https://www.who.int/news/item/06-07-2021-new-recommendations-for-screening-and-treatment-to-prevent-cervical-cancer (accessed on 6 July 2021).
15. Fontham, E.T.; Wolf, A.M.; Church, T.R.; Etzioni, R.; Flowers, C.R.; Herzig, A.; Guerra, C.E.; Oeffinger, K.C.; Shih, Y.C.T.; Walter, L.C. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. CA A Cancer J. Clin. 2020, 70, 321–346.
16. Obermair, H.M.; Bennett, K.F.; Brotherton, J.M.; Smith, M.A.; McCaffery, K.J.; Dodd, R.H. Australian National Cervical Screening Program renewal: Attitudes and experiences of general practitioners, and obstetricians and gynaecologists. Aust. N. Z. J. Obstet. Gynaecol. 2021, 61, 416–423.
17. Brown, R.F.; Muller, T.R.; Olsen, A. Australian women's cervical cancer screening attendance as a function of screening barriers and facilitators. Soc. Sci. Med. 2019, 220, 396–402.
18. Gu, C.; Chan, C.W.; Chow, K.M.; Yang, S.; Luo, Y.; Cheng, H.; Wang, H. Understanding the cervical screening behaviour of Chinese women: The role of health care system and health professions. Appl. Nurs. Res. 2018, 39, 58–64.
19. Schiffman, M.; Wentzensen, N. A Suggested Approach to Simplify and Improve Cervical Screening in the United States. J. Low. Genit. Tract Dis. 2016, 20, 1–7.
20. von Karsa, L.; Arbyn, M.; De Vuyst, H.; Dillner, J.; Dillner, L.; Franceschi, S.; Patnick, J.; Ronco, G.; Segnan, N.; Suonio, E. European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. Papillomavirus Res. 2015, 1, 22–31.
21. ASCO. Secondary Prevention of Cervical Cancer: American Society of Clinical Oncology Resource-Stratified Clinical Practice Guideline. 2016. Available online: https://www.asco.org/sites/new-www.asco.org/files/content-files/practice-and-guidelines/documents/2016-rs-secondary-prev-cervical-slides2.pdf (accessed on 1 January 2016).
22. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J. Clin. 2021, 71, 209–249.
23. Nayar, R.; Wilbur, D.C. The Pap test and Bethesda 2014. Cancer Cytopathol. 2015, 123, 271–281.
24. Tsikouras, P.; Zervoudis, S.; Manav, B.; Tomara, E.; Iatrakis, G.; Romanidis, C.; Bothou, A.; Galazios, G. Cervical cancer: Screening, diagnosis and staging. J. BUON 2016, 21, 320–325.
25. Ronco, G.; Giorgi-Rossi, P.; Carozzi, F.; Confortini, M.; Dalla Palma, P.; Del Mistro, A.; Ghiringhello, B.; Girlando, S.; Gillio-Tos, A.; De Marco, L.; et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: A randomised controlled trial. Lancet Oncol. 2010, 11, 249–257.
26. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2020. CA Cancer J. Clin. 2020, 70, 7–30.
27. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424.
28. Monsonego, J. Global challenges of cervical cancer prevention. Eur. J. Gynaec. Oncol.-IssN 2000, 392, 2936.
29. De Martel, C.; Plummer, M.; Vignat, J.; Franceschi, S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int. J. Cancer 2017, 141, 664–670.

30. Pirog, E.C. Cervical adenocarcinoma: Diagnosis of human papillomavirus–positive and human papillomavirus–negative tumors. Arch. Pathol. Lab. Med. 2017, 141, 1653–1667.
31. Holl, K.; Nowakowski, A.M.; Powell, N.; McCluggage, W.G.; Pirog, E.C.; Collas De Souza, S.; Tjalma, W.A.; Rosenlund, M.; Fiander, A.; Castro Sánchez, M. Human papillomavirus prevalence and type-distribution in cervical glandular neoplasias: Results from a E uropean multinational epidemiological study. Int. J. Cancer 2015, 137, 2858–2868.
32. Stolnicu, S.; Barsan, I.; Hoang, L.; Patel, P.; Terinte, C.; Pesci, A.; Aviel-Ronen, S.; Kiyokawa, T.; Alvarado-Cabrero, I.; Pike, M.C. International Endocervical Adenocarcinoma Criteria and Classification (IECC): A new pathogenetic classification for invasive adenocarcinomas of the endocervix. Am. J. Surg. Pathol. 2018, 42, 214.
33. Tjalma, W. HPV negative cervical cancers and primary HPV screening. Facts Views Vis. ObGyn 2018, 10, 107.
34. Szarewski, A.; Ambroisine, L.; Cadman, L.; Austin, J.; Ho, L.; Terry, G.; Liddle, S.; Dina, R.; McCarthy, J.; Buckley, H. Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears. Cancer Epidemiol. Prev. Biomark. 2008, 17, 3033–3042.
35. Mayrand, M.-H.; Franco, E.L. Integrating novel primary-and secondary-prevention strategies: The next challenge for cervical cancer control. Future Oncol. 2010, 6, 1725–1733.
36. Wentzensen, N.; von Knebel Doeberitz, M. Biomarkers in cervical cancer screening. Dis. Markers 2007, 23, 315–330.
37. Cuschieri, K.; Wentzensen, N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. Cancer Epidemiol. Prev. Biomark. 2008, 17, 2536–2545.
38. Lawicki, S.; Bedkowska, E.; Gacuta-Szumarska, E.; Knapp, P.; Szmitkowski, M. The plasma levels and diagnostic utility of stem cell factor (SCF) and macrophage-colony stimulating factor (M-CSF) in cervical cancer patients. Pol. Merkur. Lek. 2008, 25, 38–42.
39. Murphy, N.; Ring, M.; Heffron, C.C.; King, B.; Killalea, A.G.; Hughes, C.; Martin, C.M.; McGuinness, E.; Sheils, O.; O'Leary, J.J. p16INK4A, CDC6, and MCM5: Predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. J. Clin. Pathol. 2005, 58, 525–534.
40. Gajer, P.; Brotman, R.M.; Bai, G.; Sakamoto, J.; Schutte, U.M.; Zhong, X.; Koenig, S.S.; Fu, L.; Ma, Z.S.; Zhou, X.; et al. Temporal dynamics of the human vaginal microbiota. Sci. Transl. Med. 2012, 4, 132ra152.
41. Ravel, J.; Gajer, P.; Abdo, Z.; Schneider, G.M.; Koenig, S.S.; McCulle, S.L.; Karlebach, S.; Gorle, R.; Russell, J.; Tacket, C.O.; et al. Vaginal microbiome of reproductive-age women. Proc. Natl. Acad. Sci. USA 2011, 108 (Suppl. S1), 4680–4687.
42. Mitra, A.; MacIntyre, D.A.; Marchesi, J.R.; Lee, Y.S.; Bennett, P.R.; Kyrgiou, M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: What do we know and where are we going next? Microbiome 2016, 4, 58.
43. Kalof, A.N.; Cooper, K. p16INK4a immunoexpression: Surrogate marker of high-risk HPV and high-grade cervical intraepithelial neoplasia. Adv. Anat. Pathol. 2006, 13, 190–194.
44. Trunk, M.J.; Dallenbach-Hellweg, G.; Ridder, R.; Petry, K.U.; Ikenberg, H.; Schneider, V.; von Knebel Doeberitz, M. Morphologic characteristics of p16INK4a-positive cells in cervical cytology samples. Acta Cytol. 2004, 48, 771–782.
45. Schmidt, D.; Bergeron, C.; Denton, K.J.; Ridder, R.; European, C.C.S.G. p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL papanicolaou cytology: Results from the European equivocal or mildly abnormal Papanicolaou cytology study. Cancer Cytopathol. 2011, 119, 158–166.
46. Bergeron, C.; Ikenberg, H.; Sideri, M.; Denton, K.; Bogers, J.; Schmidt, D.; Alameda, F.; Keller, T.; Rehm, S.; Ridder, R.; et al. Prospective evaluation of p16/Ki-67 dual-stained cytology for managing women with abnormal Papanicolaou cytology: PALMS study results. Cancer Cytopathol. 2015, 123, 373–381.
47. Kruse, A.J.; Baak, J.P.; de Bruin, P.C.; Jiwa, M.; Snijders, W.P.; Boodt, P.J.; Fons, G.; Houben, P.W.; The, H.S. Ki-67 immunoquantitation in cervical intraepithelial neoplasia (CIN): A sensitive marker for grading. J. Pathol. 2001, 193, 48–54.
48. FDA. Premarket Approval (PMA). 2020. Available online: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P190024 (accessed on 3 October 2020).
49. Peeters, E.; Wentzensen, N.; Bergeron, C.; Arbyn, M. Meta-analysis of the accuracy of p16 or p16/Ki-67 immunocytochemistry versus HPV testing for the detection of CIN2+/CIN3+ in triage of women with minor abnormal cytology. Cancer Cytopathol. 2019, 127, 169–180.
50. Wright, T.C., Jr.; Behrens, C.M.; Ranger-Moore, J.; Rehm, S.; Sharma, A.; Stoler, M.H.; Ridder, R. Triaging HPV-positive women with p16/Ki-67 dual-stained cytology: Results from a sub-study nested into the ATHENA trial. Gynecol. Oncol. 2017, 144, 51–56.

51. Petry, K.U.; Schmidt, D.; Scherbring, S.; Luyten, A.; Reinecke-Luthge, A.; Bergeron, C.; Kommoss, F.; Loning, T.; Ordi, J.; Regauer, S.; et al. Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology. Gynecol. Oncol. 2011, 121, 505–509.
52. Wentzensen, N.; Schwartz, L.; Zuna, R.E.; Smith, K.; Mathews, C.; Gold, M.A.; Allen, R.A.; Zhang, R.; Dunn, S.T.; Walker, J.L.; et al. Performance of p16/Ki-67 immunostaining to detect cervical cancer precursors in a colposcopy referral population. Clin. Cancer Res. 2012, 18, 4154–4162.
53. Yu, L.L.; Chen, W.; Lei, X.Q.; Qin, Y.; Wu, Z.N.; Pan, Q.J.; Zhang, X.; Chang, B.F.; Zhang, S.K.; Guo, H.Q.; et al. Evaluation of p16/Ki-67 dual staining in detection of cervical precancer and cancers: A multicenter study in China. Oncotarget 2016, 7, 21181–21189.
54. Tay, T.K.Y.; Lim, K.L.; Hilmy, M.H.; Thike, A.A.; Goh, S.T.; Song, L.H.; Hwang, J.S.G.; Mantoo, S. Comparison of the sensitivity and specificity of p16/Ki-67 dual staining and HPV DNA testing of abnormal cervical cytology in the detection of histology proven cervical intraepithelial neoplasia grade 2 and above (CIN 2+). Malays J. Pathol. 2017, 39, 257–265.
55. Possati-Resende, J.C.; Fregnani, J.H.; Kerr, L.M.; Mauad, E.C.; Longatto-Filho, A.; Scapulatempo-Neto, C. The Accuracy of p16/Ki-67 and HPV Test in the Detection of CIN2/3 in Women Diagnosed with ASC-US or LSIL. PLoS ONE 2015, 10, e0134445.
56. Hammes, L.S.; Tekmal, R.R.; Naud, P.; Edelweiss, M.I.; Kirma, N.; Valente, P.T.; Syrjanen, K.J.; Cunha-Filho, J.S. Up-regulation of VEGF, c-fms and COX-2 expression correlates with severity of cervical cancer precursor (CIN) lesions and invasive disease. Gynecol. Oncol. 2008, 110, 445–451.
57. Kirma, N.; Hammes, L.S.; Liu, Y.G.; Nair, H.B.; Valente, P.T.; Kumar, S.; Flowers, L.C.; Tekmal, R.R. Elevated expression of the oncogene c-fms and its ligand, the macrophage colony-stimulating factor-1, in cervical cancer and the role of transforming growth factor-beta1 in inducing c-fms expression. Cancer Res. 2007, 67, 1918–1926.
58. Lawicki, S.; Bedkowska, G.E.; Gacuta-Szumarska, E.; Knapp, P.; Szmitkowski, M. Pretreatment plasma levels and diagnostic utility of hematopoietic cytokines in cervical cancer or cervical intraepithelial neoplasia patients. Folia Histochem. Cytobiol. 2012, 50, 213–219.
59. Zajkowska, M.; Zbucka-Kretowska, M.; Sidorkiewicz, I.; Lubowicka, E.; Gacuta, E.; Szmitkowski, M.; Chrostek, L.; Lawicki, S. Plasma levels and diagnostic utility of macrophage-colony stimulating factor, matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 as tumor markers in cervical cancer patients. Tumour. Biol. 2018, 40, 1010428318790363.
60. Lubowicka, E.; Zbucka-Kretowska, M.; Sidorkiewicz, I.; Zajkowska, M.; Gacuta, E.; Puchnarewicz, A.; Chrostek, L.; Szmitkowski, M.; Lawicki, S. Diagnostic Power of Cytokine M-CSF, Metalloproteinase 2 (MMP-2) and Tissue Inhibitor-2 (TIMP-2) in Cervical Cancer Patients Based on ROC Analysis. Pathol. Oncol. Res. 2020, 26, 791–800.
61. Sidorkiewicz, I.; Zbucka-Kretowska, M.; Zareba, K.; Lubowicka, E.; Zajkowska, M.; Szmitkowski, M.; Gacuta, E.; Lawicki, S. Plasma levels of M-CSF and VEGF in laboratory diagnostics and differentiation of selected histological types of cervical cancers. BMC Cancer 2019, 19, 398.
62. Atoe, K.; Ehimigbai, R.; Ayinbuomwan, E.; Onovughakpo-Sakpa, O. Assessing the utility of a cytokine in the diagnosis of cervical intraepithelial neoplasm among women in Benin city, Nigeria. Afr. J. Health Saf. Environ. 2020, 1, 101–112.