Two Landmark Studies Published in 1976/1977 Paved the Way for the Recognition of Human Papillomavirus as the Major Cause of the Global Cancer Burden

Kari J. Syrjänen

Department of Clinical Research, Biohit Oyj, Helsinki, Finland; Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, Brazil

Keywords

Koilocyte · Pap smear · Human papillomavirus · Genital wart · Cervical cancer · Dysplasia · Cervical intraepithelial neoplasia · Causal agent · Histology · Viral cytopathic change · Oncogenic virus · Global burden · Prophylactic vaccines

Abstract

Two groundbreaking reports were published in *Acta Cytologica* at the transition of 1976 to 1977. One appeared in the last issue of 1976 [Meisels and Fortin: *Acta Cytol* 1976;20: 505–509] and the other in the first issue of 1977 [Purola and Savia: *Acta Cytol* 1977;21:26–31]. Today, 40 years later, it is not an overstatement to conclude that these are the two most influential studies ever published in this journal. Two reports with a similar content being published so close together (in the same journal) raised the question “Which of the two reports was truly submitted first?” In this commentary, this enigma is clarified beyond reasonable doubt, based on the well-considered testimonial of Prof. Leopold G. Koss, the reviewer of one of the two papers. To fully appreciate the significance of the novel discovery made in these two re-

ports, it is essential to align them in the right context, both retrospectively and prospectively. This commentary will assist the reader by summarizing the existing knowledge on human papillomavirus (HPV) before these two milestone papers appeared, and describe the incredibly rapid progress that they evoked during the subsequent decades, which made HPV the single most important human tumor virus. As the final proof of virus-cancer causality, prophylactic HPV vaccines have been effective in preventing (a) virus transmission and HPV infection, (b) benign HPV-induced tumors (genital warts), and (c) cervical intraepithelial neoplasia (CIN). Formal evidence of the prevention of cervical cancer by these HPV vaccines still awaits confirmation, and the same applies to the eventual prevention of human cancers at other anatomic sites, part of the global burden of oncogenic HPVs.

© 2017 S. Karger AG, Basel

Background

It is a great privilege to write a commentary on two studies that appeared 40 years ago (in 1976/1977) that are, beyond doubt, the two most influential studies ever

published in *Acta Cytologica* during its entire 60-year life span. These two studies, by Meisels and Fortin [1] and Purola and Savia [2], were the first to recognize that dysplastic (the cancer precursor, cervical intraepithelial neoplasia [CIN]) lesions in the uterine cervix are of viral origin, thus introducing the concept that human papillomavirus (HPV) might be the etiological agent of cervical cancer. This groundbreaking new concept paved the way for the emergence of HPV research as one of the most rapidly developing fields of oncology, as witnessed during these past four decades, and culminating in the Nobel Prize in Medicine and Physiology being granted to Prof. Harald zur Hausen in 2008. HPV is among the first human tumor viruses for which all six of Koch's postulates, required for proof of causality (i.e., HPV is the cause of cancer), have been fulfilled. The final proof is the recent demonstration that prophylactic HPV vaccines are indeed effective in preventing (a) viral transmission and HPV infection, (b) benign HPV-induced tumors (genital warts), and (c) CIN, albeit that the evidence of the prevention of cervical cancer by these HPV vaccines still awaits confirmation [3]; the same is true of the eventual prevention of human cancers at other anatomic sites, part of the global burden of oncogenic HPVs [4].

All novel observations and ideas in medical research are based on the previous discoveries made by other scientists (contemporary or past). To fully appreciate the importance of each novel discovery, it is essential to be able to place them in the right context, both retrospectively and prospectively. This commentary will assist the reader by summarizing the existing knowledge on HPV before these two milestone papers appeared [1, 2], and describing the incredibly rapid progress that they evoked during the subsequent years and decades. Before this, however, the delicate issue as to which of the two was truly submitted first is discussed, an issue inevitably provoked by the close proximity of the publication of two reports with an obviously identical content in the same journal.

Which of the Two Papers Was Truly the First?

It might sound trivial after 40 years to consider the questions: (1) Which of these two reports [1, 2] was submitted first? (2) Did the submission of two papers with a practically identical content truly happen without awareness of each other? (3) Or is only one of them the original and the other one simply a replica?

When two study groups are reporting similar results that are of low or even moderate importance, one would consider such a discussion as being of academic relevance only. This case is different, however, due to the dramatic impact that these two reports had on the subsequent progress in human cancer research that, in less than 3 decades, permanently changed our concepts about the contribution of human tumor viruses to the global cancer burden. Beyond doubt, this development would have been the same, irrespective which of the two studies was published first, and even in the case of only one of them being published in 1976/1977. Thus, from this point of view, this issue appears to be of secondary importance.

Because this controversy was publicly addressed much later by others (most notably by Leopold G. Koss, but in a document that might have remained unnoticed from the majority of our regular readers), this enigma will be briefly addressed here as an example of how several things went wrong 40 years ago. In the history of clinical cytology, however, this is not the only occasion when such things happen, as pointed out by Dr. Koss in his thoughtful commentary on milestone articles [5].

Referring back to the contention over the reports [1, 2], the most comprehensive treatise on this subject can be found in a book written in 2004 by Dr. Ervo Vesterinen [6], who was a close collaborator of Drs. Purola and Savia for decades. This book is written in Finnish with the title "Story of the Pap Test." It contains, as the core document, the commentary of Leopold G. Koss [5], which he wrote in 2003 as a response to Dr. R.G. Lynch, whose commentary on milestone papers in cytology had appeared in the previous issue of the *Bulletin of the American Society for Investigative Pathology* [7]. In his commentary, Dr. Lynch only discussed the contribution of Meisels and Fortin [1], with no mention of the work of Purola and Savia [2].

In his unsurpassed style, commenting on Lynch's article [7], Leopold G. Koss wrote [5]:

I read, with great interest, the article in the recent *ASIP Bulletin* (vol. 6, issue 1, pages 10–11) on the topic of condylomatous lesions of the cervix and vagina that led to the study of the role of human papillomavirus in cervix cancer. It is sometimes extremely difficult to establish priorities in important scientific observations that lead to a cascade of events with unpredictable and surprising results. Without wishing to detract, in any way, from the contributions of Dr. Alexander Meisels and his colleagues that were described in the article, the priority of the observations that koilocytes and, hence, precancerous lesions of the uterine cervix, may be caused by viral infection, is attributable to the Finnish observers, Esko Purola and Eeva Savia, whose article on the topic appeared in the subsequent issue of *Acta Cytologica*, one month after the Meisels paper.

Dr. Koss continued:

Because the priority of these observations became contentious, it was subsequently acknowledged by the Editor of *Acta Cytologica* (vol. 21, page 483, 1977) that the Purola and Savia paper was submitted to the journal, *Acta Cytologica*, on May 13, 1975, seven months before the Meisels and Fortin paper, which was received on December 11, 1975. I was the fussy reviewer of the Purola and Savia paper who returned it twice to the authors for improvements. In fact, their paper is, in many ways, more explicit than the Meisels and Fortin contribution. It begins with the statement, "The viral origin of condylomata accuminata in genital warts is beyond doubt." The paper further deducts that similar lesions of the uterine cervix most likely have a viral origin. I feel badly that the delay in its publication was caused by my interference.

Leo Koss concluded his commentary by stating:

Sic transit gloria mundi.

As described by Vesterinen in the book [6], being an associate editor of *Acta Cytologica*, Dr. Meisels got his paper published without any such delay, and it appeared in the last issue of 1976 [1], while the Purola and Savia paper was suspended until the first issue of 1977 [2] (for details concerning the reasons for this delay, see [6]). Although the documents by Koss [5] and Vesterinen [6] give us a clear picture of which report was submitted first to *Acta Cytologica*, they do not give us a definite answer to questions (2) and (3) in the first paragraph under this heading. As the original authors are no longer with us, this mystery will most likely remain unanswered, and we leave it up to the discretion of the readers to judge.

What Was Known about PVs and Human Disease before 1976/1977?

The present-day understanding of HPV research and clinical practice is a result of a long history of the pioneering works of a countless number of past and contemporary scientists. The history of papillomavirus (PV) research has an interesting dual nature: (1) basic virological data emerged from animal experiments during the first half of the past century, and (2) an increasing awareness of these viruses as a significant cause of human cancer emerged in the late 1970s.

The history of PV research has attracted scientists for quite a long time, with the first reviews dating back to the early 1950s and 1960s [8, 9]. The most recent discourses on this intriguing topic are found in my textbook, published in 2000 [10], and in journal articles from 2008 [11] and 2009 [12]; albeit with a slightly different focus, the significant historical milestones in PV research are listed

quite consistently in all of these reviews. The focus of this communication is to describe the impact that the two milestone papers [1, 2] have had in the progress of PV science, but it is not feasible to discuss all the historical details from before 1976/1977. To help the orientation of the reader, however, these are listed in Table 1.

From these data, it is obvious that, particularly during the 1970s, the research rapidly progressed towards an understanding of the plurality of HPV and its important role as a human tumor virus (Table 1). The 1970s was heralded by emerging data on the serological response to human wart viruses (HPV). In this respect, the pioneering observations of a Finnish virologist, Seppo Pyrhönen and his colleagues have received far too little attention. They were the first to detect measurable antibodies against pooled, homogenized human wart tissue, detected in 92% of women and 75% of men aged between 15 and 19 years [13]. Their observation on a roughly inverse correlation between the number and duration of skin warts and the antibody levels was also important [13, 14]. While studying patients with systemic lupus erythematosus, who showed HPV antibodies less frequently than their immunocompetent counterparts, the authors concluded that this lack of humoral response to HPV might explain the increased frequency of warts in these patients [15]. They also speculated on the antigenic relatedness between the HPVs in genital and skin warts, as suggested by the less frequent occurrence of HPV antibodies in patients with genital warts only. While detecting measurable HPV antibodies in students reporting not ever having had visible wart lesions, they made the assumption that subclinical HPV infections must be quite common. They also concluded that there must be more than one HPV type [15, 16].

Beyond doubt, the HPV research of today owes much to one of the pioneers in the field, Harald zur Hausen, who turned his interest from HSV to HPV in the early 1970s. In the first of his classical series of four works from 1974–1976, attempting to detect virus-specific DNA in human tumors, he completed nucleic acid hybridizations with the complementary RNA of the human wart virus [17]. It soon became evident that different viruses are responsible for cutaneous common warts and genital warts. He also formulated the hypothesis on HPV as the etiological agent of cervical cancer [18, 19], most comprehensively presented in his classical review of 1977 [20].

Thus, by 1976/1977, the time had ripened to translate this virological knowledge to the clinic, and this is exactly what Purola and Savia and Meisels and Fortin did in their reports. One cannot avoid an impression, however, that

Table 1. Key discoveries signifying milestones in papillomavirus research before 1976/1977

Year	Authors [ref.]	Discovery
460–377 BC	Hippocrates	The name “condyloma” endowed
25 AD	Celcus	Three types of skin warts: acrochordon, thymion, myrmecia
1842	Rigoni-Stern	“Venereal” nature of cervical cancer: rare in virgins and nuns
1891	Payne	Infectious nature of common warts
1901	Heidingsfield	Oral condylomas: a sign of illicit sex
1907	Ciuffo	Demonstration of the viral nature of skin warts
1923	Ullman	Demonstration of the viral nature of laryngeal papillomas
1933	Shope and Hurst	Isolation and characterization of cottontail rabbit papillomavirus (CRPV)
1935	Rous and Beard	Development of rabbit skin carcinomas due to synergistic actions between CRPV and chemical carcinogens
1938	Kidd and Rous	
1944	Friedewald and Rous	
1942–1943	Parsons and Kidd	Natural history of rabbit oral papillomas as a viral disease
1949	Ayre [81]	Halo cells in cervical carcinogenesis
1949	Strauss et al.	HPV particles in skin warts on electron microscopy (TEM)
1950	Syverton et al. [8]	Papilloma-to-carcinoma sequence in cottontail rabbits
1951	Olson et al.	Equine sarcoids caused by an agent of bovine papilloma (actually BPV)
1954	Barrett and Silbar	Genital warts shown to be a sexually transmitted disease (STD)
1956	Koss and Durfee [82]	The concept of koilocytosis in Pap smears and cervical biopsies
1957	Jablonska et al.	Revival of epidermodysplasia verruciformis (EV) as a viral disease
1961	Ito and Evans	Induction of papillomas in domestic rabbits by CRPV
1965	Crawford, Klug and Finch	Structural and molecular characterization (mw) of papillomavirus DNA extracted from skin warts
1962	Melnick	Human papova virus group (new classification)
1967	Rawson and Mahy	
1968	Dunn and Ogilvie [31]	Demonstration of viral particles in genital warts on TEM
1969	Almeida et al. [32]	Characterization of these viral particles as papova viruses
1972–1975	Pyrhönen et al. [13–16]	Series of studies on antibody response to cutaneous and genital warts
1974	zur Hausen et al. [17]	
1975–1976	zur Hausen [18, 19]	Hypothesis presented on HPV as the potential cause of cervical cancer
1976	Gissmann and zur Hausen [21]	
1977	Orth et al. [23]	

they did this unintentionally, because none of the preceding virological (molecular) studies from the past decades (Table 1), nor the more recent ones by zur Hausen [17–19] and Pyrhönen [13, 14], are included in their references or discussed to any extent.

Evidence on HPV as the Most Important Tumor Virus Started Emerging in 1976/1977

The period 1976/1977 was unrivalled in the history of HPV research in many respects, not only because of the two abovementioned contributions, but also because of the major progress made in basic research, most notably by the groups of Harald zur Hausen (Freiburg, West Ger-

many) and Gerard Orth (Paris, France). In a series of studies, these two groups established the plurality of HPV, by disclosing and characterizing the first 4 HPV types in skin warts, numbered HPV1–4 [21–23]. Serological evidence was soon provided to support the concept of plurality [15, 16], because there seemed to be no link between HPV1–4 and the new HPV type (to be characterized later as HPV6) found in condylomata acuminata (CA), laryngeal papilloma, or any of the malignant squamous cell lesions tested [24].

From the clinical point of view, the major breakthrough was indeed provided by the reports of Purola and Savia and Meisels and Fortin [1, 2], by describing the koilocytotic cells in cervical Pap smears derived from flat epithelial lesions that are frequently associated with cervi-

cal precancer lesions. In addition to these characteristic koilocytes, they accurately described two other cell types that regularly exfoliate from genital CA: dyskeratotic superficial cells and “condylomatous” intermediate cells. They were the first to realize that by observing the cytopathic effects of a virus under a light microscope, one could probably “see” the evidence for an etiological agent of cervical cancer and its precursor lesions on biopsies and Pap smears.

Like most novel innovations, it took quite a while until this revolutionary idea became more generally accepted among cytopathologists. I was possibly one of the first to realize the significance of this observation. I therefore have deep personal feelings when speaking about these innovative reports, discussed in an issue of *CytoPaths* [25]. In fact, February 1977 was my first month as Resident at the Department of Pathology, Jorvi Hospital (now Helsinki University Hospital), and the two most recent issues of *Acta Cytologica* were brought to my attention as “recommended reading” by my tutors and mentors. Having familiarized myself with the idea, as a young man with little experience in clinical pathology, I became curious enough to start collecting all the Pap smears and biopsies of cervical cancer and precancer lesions from the Department archives. The work progressed slowly, however, because the hospital was brand new (<1 year old) and not many cases had accumulated. Finally, however, enough cases were available ($n = 184$) to make the analysis, which fully confirmed the observations of Meisels and Fortin and Purola and Savia [1, 2]. Illustrative of this is the fact that, a report published in 1979 in *Archives of Gynecology* [26] was the first to reproduce the original findings in a similar setting of the light-microscopic examination of cervical precancer lesions. Even the next year (1980), when my first review on the subject was published, my reference list was very scanty indeed, mostly comprising older and more recent virological studies [27]. Well before that, however, we submitted another cytological study to *Acta Cytologica*, which was readily accepted but, at the time, the journal was so crowded with papers that it took almost two full years to get it published [28].

To make the 40-year history of PV/HPV research more tangible, Table 2 lists the key discoveries made since that unrivalled time in 1976/1977. A short note follows here of all the cited discoveries so as to better cover the title of this commentary.

Della Torre et al. [29] and Hills and Laverty [30] were the first to detect viral particles within the nuclei of koilocytes on transmission electron microscopy (TEM). These viral particles were identical to those described in CA in

the late 1960s [31, 32], and in skin even earlier, in 1949 [33]. This confirmed that these newly described flat and endophytic epithelial lesions of the uterine cervix are manifestations of HPV, although distinct from the classical CA in their light-microscopic appearance. The circle was completed in 1979, when an invasive cervical squamous cell carcinoma (SCC) was described with an abundance of koilocytes in the biopsies [34].

In 1978, two new HPV types associated with epidermodysplasia verruciformis (EV) lesions were discovered by Orth et al. [35], paving the way for the subsequent detection of a large number of the so-called “EV-specific” HPV types recognized today. In parallel with the incited interest in HPV lesions of the genital tract and skin, the suspected HPV origin of two additional lesions was confirmed: (1) juvenile-onset laryngeal papilloma, and later (2) adult-onset laryngeal papilloma. Quick et al. [36, 37] described epithelial atypia in these lesions, with the possible implications of their known risk for malignant transformation. Soon, conclusive clinical and virological evidence on similarities between genital condylomas and laryngeal papillomas was provided [38]. Within the next two years, HPV involvement in laryngeal SCC was suggested, based on the morphological features and detection of HPV antigens by immunohistochemistry (IHC) in up to 36% of the 116 cases [39, 40]. When subjected to meta-analysis in 2016, the literature worldwide included 179 studies, comprising 7,437 laryngeal SCCs analyzed, with a pooled HPV prevalence of 26.9% (95% CI 24.2–29.7%; random-effects model) [41].

Before the introduction of DNA technology for general use, morphology, TEM and IHC were the diagnostic tools to provide evidence for HPV involvement in genital and extragenital squamous cell lesions. Prompted by the encouraging findings in laryngeal SCC, we focused our interest on similar lesions in the lower respiratory tract, i.e., bronchial SCC [42]. Using these simple means, we described koilocytotic cells and other morphological evidence (confirmed by IHC) suggestive of HPV involvement in bronchial SCCs, first in a single case [42], and subsequently in a series of 104 SCCs, 34.6% of which were interpreted as suggestive of HPV [43]. These observations paved the way to a more widespread thinking about HPV as the potential etiological agent in human SCCs at mucosal sites other than the genital tract and skin. These pioneering data seem to have withstood the test of time, as suggested by a meta-analysis published 30 years later (in 2012), where 100 eligible studies with 7,381 lung cancers showed a pooled HPV prevalence of 34.8% (95% CI 33.3–36.3%) [44].

Table 2. Key discoveries signifying milestones in papillomavirus research since 1976/1977

Year	Authors [ref.]	Discovery
1976	Meisels and Fortin [1]	Description of koilocytotic atypia as the cytopathic sign of HPV in flat
1977	Puroola and Savia [2]	condylomas and dysplastic lesions of the uterine cervix
1978	Pyrhönen and Neuvonen [16]	Wart (papilloma) virus antibodies detected in dog, pigs and cattle
1978	Della Torre et al. [29]	Demonstration of HPV particles in dysplastic lesions of the uterine
1979	Hills and Lavery [30]	cervix by TEM
1978	Orth et al. [35]	Specific HPV types in skin carcinomas derived from EV
1978–1980	Quick et al. [36–38]	Classical works on laryngeal papillomas and their association with HPV
1979	Syrjänen [34]	Koilocytotic cells in invasive carcinoma of the uterine cervix
1979	Law et al. [45]	Gene cloning and HPV hybridization under non-stringent conditions
1979	Syrjänen [42]	HPV-suggestive cytopathic changes detected in bronchial SCC
1979	Coggin and zur Hausen [46]	System for classification of HPVs
1980	Gissmann and zur Hausen [49]	Characterization of HPV6 from a condyloma acuminatum lesion
1981	de Villiers et al. [50]	Cloning of HPV6 in a bacterial vector
1982	Gissmann et al. [51]	Cloning and characterization of HPV11 from a laryngeal papilloma
1981	Syrjänen et al. [53]	The first prospective follow-up study on cervical HPV lesions started
1982	Syrjänen [59]	HPV involvement in esophageal papilloma and squamous cell cancer
1983	Syrjänen et al. [60]	HPV involvement in sinonasal papilloma/carcinoma
1983	Dürst et al. [52]	Characterization of HPV16 from a cervical carcinoma
1982	Jenson et al. [63]	First evidence on HPV involvement in both benign and malignant
1983	Syrjänen et al. [64]	lesions of the oral mucosa
1984	Boshart et al. [54]	Characterization of HPV18 from a cervical carcinoma
1985	Kreider et al. [69]	Condyloma lesions induced by HPV11 keratinocytes grafted in nude mice
1985	Beckman et al. [70]	Technique of HPV in situ hybridization (ISH) described in biopsies
	Gupta et al. [71]	
1986	Lorincz et al. [55]	Characterization of HPV31
1986	Beaudenon et al. [56]	Characterization of HPV33
1986	Yasumoto et al. [74]	Malignant transformation of NIH3T3 cells by HPV16
1987	Pirisi et al. [75]	Immortalization of human keratinocytes by HPV16
	Dürst et al. [76]	
1988	McCance et al. [77]	Organotypic raft culture as in vitro model for HPV research
1989	Dyson et al. [78]	HPV E7 binding with the pRb protein
1990	Werness et al. [79]	HPV E6 binding with the p53 protein
1992	Kirnbauer et al. [80]	Production of virus-like particles (VLP) by HPV L1 and L2 self-assembly
2006	Merck	FDA licensed a quadrivalent HPV vaccine, Gardasil® (HPV types 6, 11, 16, 18)
2007	GSK	FDA licensed a bivalent HPV vaccine, Cervarix® (HPV6, HPV11)
2014	Merck	FDA approved of a 9-valent HPV vaccine, GARDASIL®9 (HPV types 6, 11, 16, 18, 31, 33, 45, 52, 58)

Mandatory DNA techniques also developed fast. Dr. Howley's group [45] was the first to adopt gene cloning and hybridization under nonstringent conditions for PV research, showing the highly conserved nucleotide sequences in PVs. The decade was concluded by an introduction of the first unanimously agreed classification of PVs, based on the decisions of the First Workshop on

Papillomaviruses [46]. This first classification was based exclusively on the sequence homology between the different PV isolates: if there was a <50% crosshybridization with the known PV types, the viral DNA was assigned a new type. This classification prevailed until the 1990s, when it was revised, and finally, in 2004, PVs were classified as a taxonomic family of their own [47].

The Speed of Progress Increases throughout the 1980s

The 1980s witnessed incredible and dramatic progress in all fields of PV research, mostly because of the development of molecular cloning and related techniques [45]. This speed is best illustrated by the rapid discovery, cloning, and characterization of an ever-growing number of new HPV types, for which there is no end in sight, even today [48]. This development was started in 1980 by Gissmann and zur Hausen [49], who isolated, characterized, and cloned a new virus, which proved to be the etiological agent of classical CA and was designated as HPV6 [50]. Characterization of the first of these genital HPV types led to the isolation of its closest relative from laryngeal papilloma receiving the label HPV11 [51]. All attempts to detect homologous DNA in laryngeal SCCs failed, however [51].

One of the absolute highlights was the isolation and characterization of a new HPV type from cervical cancer by Dürst et al. [52] in 1983, which has subsequently proved to be the single most important HPV type of all, namely HPV16. While tested in a series of biopsies from cervical, vulvar, and penile cancer, >60% of cervical cancer samples were found to hybridize with HPV16 DNA, the corresponding figures for vulvar and penile cancer being 28.6 and 25%, respectively. In contrast, practically none of the benign CA lesions were shown to contain HPV16 DNA, which led the authors to suggest that HPV16 is an HPV type characteristic of SCC of the genital tract. This was soon confirmed in a prospective study, where HPV16 showed an extremely high propensity for malignant transformation [53].

In 1984, HPV18 was isolated and characterized from cervical carcinoma [54]. Importantly, HPV18 DNA was also found in several cell lines derived from cervical cancer, including the HeLa, KB, and C4-1 lines [54]. In 1986, Lörincz et al. [55] isolated HPV31, and HPV33 was molecularly cloned and characterized by a French study group in the same year [56].

Although it may appear egocentric to include too many of my own contributions in this list, it is difficult to avoid mentioning October 1981, which signified the starting point of the first prospective follow-up (cohort) study of women with cervical HPV infection (1981–1998 in Kuopio, Finland). By 1985, we had established that the natural history of cervical HPV lesions was identical with that of classical CIN lesions. In the same year, the inherent potential of HPV16 and HPV18 lesions to progress to invasive cancer was firmly confirmed in our cohort [53]. The first epidemiological study on the risk factors of gen-

ital HPV infections appeared at the same time, confirming an early onset of sexual activity, the number of sexual partners, mode of contraception as well as smoking to be key risk factors for HPV infections [57].

Even if running the prospective cohort study was our main effort, our group did not lose interest in exploring the potential HPV involvement of SCC at other anatomical sites. This search led to a description of such evidence in two distinct entities, squamous cell papilloma and carcinoma of the esophagus in 1982 [58, 59], followed a year afterwards by yet another squamous cell lesion, inverted papilloma of the nasal cavity/paranasal sinuses [60]. The latter represent relatively rare tumors, but are of clinical importance due to their frequent recurrence and a definite risk for malignant transformation. Assessed 30 years afterwards by meta-analysis of the published literature, both these observations [58–60] have gained widespread acceptance in the scientific community. Indeed, esophageal SCC is the most intensely studied extragenital HPV manifestation, >10,000 samples of which have been analyzed in over 150 published studies [61]. Highly interestingly, the pooled HPV prevalence of 37.2% (95% CI 36.0–38.4%; fixed-effects model) is not far off 40% (24/60), as originally reported [59]. Concerning sinonasal papillomas, 76 eligible studies were published until 2012, with 1,956 cases analyzed, showing a pooled HPV prevalence of 42.1% (95% CI 35.9–48.5%) [62].

These strenuous years also witnessed the extension of clinical HPV research into yet another group of squamous cell lesions that subsequently gained a substantial clinical importance, i.e., the first evidence of HPV involvement in benign [63], premalignant, and malignant [64] SCCs of the oral mucosa. At the time, it proved to be extremely difficult to get the reports on such a new idea (i.e., HPV as an etiological agent of oral cancer) accepted in any journals of oral medicine, which resulted in a marked delay in the publication of these two pioneering studies [64, 65]. During the past 30 years, however, HPV as the causal factor of a subset of head and neck cancer has become an accepted fact [66, 67], and this is the first specific group of human cancers where HPV testing has become a routine practice, because it is shown to have a marked impact in a patient's treatment and survival [68].

The mid 1980s also generated significant achievements in the methodological development of HPV research. Two innovations deserve a mention: (1) the design of the so-called Kreider model [69], and (2) the development of different hybridization methods, *in situ* hybridization (ISH) being particularly innovative [70, 71]. In 1985, Kreider et al. [69] conducted experiments where they succeeded in

inducing squamous cell lesions consistent with CA in epithelial cells derived from a normal uterine cervix after exposure to HPV11 from a CA lesion. This was the first demonstration of a morphological transformation of human tissues with HPV under experimental conditions. The only disadvantage of the model was the failure of the grafted epithelium to support the replication of the oncogenic HPV16 and HPV18. ISH, developed simultaneously by two independent groups in 1985 proved to be well accepted among pathologists, because, for the first time, it allowed a direct visualization of the hybridization signals in the epithelial cells, on both biopsies and Pap smears [70, 71]. The originally used radioactive label was soon replaced by a biotin label which further increased the acceptability of the ISH technique [72].

PV/HPV research had made such remarkable progress that it was timely to publish the first textbook on the subject that would share the current state of the art with a wider readership. This project was realized in 1985–1987, when I had the privilege to work with two giants in HPV research (Lutz Gissmann and Leopold G. Koss) as the editors of a textbook, all 17 chapters of which were written by the foremost PV/HPV scientists. In 1987, once published, this first textbook on PVs became the standard reference for several years [73].

Towards Understanding the Molecular Mechanisms of HPV-Induced Cell Transformation

In parallel with the major advances made in research technology, the basic understanding of the molecular mechanisms of how PVs induce malignant transformation increased substantially via several innovative experiments. In 1986, Yasumoto et al. [74] described the transformation of a rodent cell line (NIH3T3) by HPV16. Using a recombinant HPV16 DNA (pSHPV16d), which contains a head-to-tail dimer of the full-length HPV16 genome, they could induce morphologic transformation, and, indeed, the transformed cells proved to be tumorigenic in nude mice. Subsequently, the transformation of NIH3T3 cells has provided a useful model for analyzing the functions of HPV16.

Equally important are reports from 1987 by Pirisi et al. [75] and Dürst et al. [76], where human keratinocytes and fibroblasts isolated from foreskin were transformed by transfection with recombinant HPV16 DNA. The transformed cells exhibited an extended (fibroblasts) or indefinite (keratinocytes) life span compared with that of normal controls. Such immortalized cell lines represented a

unique system to study the interaction of HPV with its natural target cell in vitro.

Yet another technical innovation, albeit not an original development, was made in 1988, when the organotypic-raft culture was modified for HPV research in Dr. Laimins' laboratory [77]. Using a cell culture system for keratinocytes which allows the stratification and production of differentiation-specific keratins, the authors examined the effects of HPV16 on the differentiation capabilities of human epithelial cells. Not unexpectedly, the histological abnormalities induced by HPV16 closely mimicked those seen in CIN lesions.

While approaching the 1990s, two key discoveries in basic HPV research deserve their place on the list, because they contributed significantly to our understanding of the basic mechanisms of virus-host cell interactions. In 1989, prompted by the previous observations that the *Rb1* (retinoblastoma susceptibility) gene product, p105Rb, forms stable complexes with the oncoproteins of the adenovirus E1A protein and the SV40 large T antigen, Dyson et al. [78] demonstrated that the HPV16 E7 oncoprotein can form similar complexes with p105Rb. While a similar mechanism in transformation is used by these three DNA viruses, this finding strongly implicated *Rb*-binding as a key molecular event in HPV-induced carcinogenesis.

This discovery was followed by a logical approach to explore if another tumor suppressor gene, p53, and its protein product will bind to other oncoproteins of HPVs. In the same year (1989), accordingly, Werness et al. [79] showed that the E6 protein of HPV16 is capable of binding to the cellular p53 protein. Because of the fact that the wild-type p53 protein also forms complexes with the SV40 large T antigen and the E1B 55 kD protein of adenovirus type 5, an analogy was established between E7-Rb and E6-p53 complexing. Undoubtedly, this was strong evidence in favor of the concept that HPVs, adenoviruses, and SV40 utilize similar cellular pathways in their cell transformation [78, 79]. Since these experiments, more than 20 human proteins have been identified as interacting with either HPV16 E6 or E7 proteins [10–12].

A Straightforward but Uneasy Track to the First Prophylactic HPV Vaccines

In 1992, a highly innovative discovery was made which subsequently opened up completely new perspectives on two important areas of HPV research: (i) serology and (ii) vaccination. This was the description of the technique for producing virus-like particles (VLP) in vitro by Kirnbau-

er et al. [80], who succeeded in expressing the L1 major capsid proteins of BPV1 and HPV16 in insect cells (using a baculovirus vector), and analyzing their conformation and immunogenicity. The L1 proteins were expressed at high levels and, surprisingly, assembled into structures that closely resembled PV virions in appearance [80]. These self-assembled BPV L1 VLPs also mimicked intact BPV virions functionally, while being capable of inducing neutralizing antibodies in rabbits. Thus, the L1 protein seems to have an intrinsic capacity to assemble into empty capsid-like structures with immunogenicity similar to that of infectious viral particles. This novel L1 VLP preparation was immediately recognized as a potential candidate for serological tests to measure antibodies to conformational virion epitopes as well as for developing a vaccine to prevent HPV infections [80].

Subsequent experimental and human studies on these cell lines resulted in the development of the first generation of prophylactic vaccines against the HPV types 6, 11, 16, and 18 (Gardasil[®], Merck) in 2006, and against HPV16 and HPV18 (Cervarix[®], GSK) in 2007. The vast literature accumulated on prophylactic HPV vaccines during the past 10 years falls outside the scope of this paper (please refer to reviews on the topic [3]).

Future Expectations of HPV Prophylaxis

The latest development in the field of prophylactic HPV vaccines represents the 9-valent GARDASIL[®]9 which obtained FDA approval in 2014 [3], and is indicated for females 9–26 years of age for the prevention of (1): cervical, vulvar, vaginal, and anal cancers caused by HPV types 16, 18, 31, 33, 45, 52, 58; (2) precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, 58; and (3) genital warts caused by HPV6 and HPV11. It is also indicated for males from 9 to 26 years of age for the prevention of: (1) anal cancer caused by HPV types 16, 18, 31, 33, 45, 52, 58; (2) precancerous or dys-

plastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, 58; and (3) genital warts caused by HPV6 and HPV11 [3]. Importantly, GARDASIL 9 does not eliminate the necessity for girls to continue to participate in cervical cancer screening later in life, and recipients of this vaccine should not discontinue anal cancer screening if this has been recommended by a health care professional.

According to the manufacturer (Merck), GARDASIL 9 has not been demonstrated to provide protection against diseases from vaccine HPV types to which a person has previously been exposed through sexual activity [3]. Similarly, this vaccine (or Gardasil and Cervarix) is not a treatment for external genital warts (CA), or cervical, vulvar, vaginal, and anal cancers and intraepithelial neoplasias. It protects only against those cervical, vulvar, vaginal, and anal cancers caused by HPV types 16, 18, 31, 33, 45, 52, and 58 [3].

With the current understanding of the role of HPVs in human carcinogenesis, and in view of the WHO incidence statistics for the 15 major malignancies worldwide, it can be estimated that oncogenic HPV types might be involved in the development of up to 10–15% of all human malignancies [4]. How much of this global cancer burden can be eventually prevented by the currently available prophylactic HPV vaccines remains to be seen during the next few decades. This possibility clearly exists already today, thanks to the astounding progress in HPV research since 1976/1977. Much of this development is indebted to the pioneering works of Purola and Savia [2] and Meisels and Fortin [1], who showed us that the same virus that was known for decades to induce benign genital warts was indeed capable of inducing squamous cell pre-cancer lesions and cancer.

Disclosure Statement

The author has no conflicts of interest to declare.

Condylomatous Lesions of the Cervix and Vagina

I. Cytologic Patterns

ALEXANDER MEISELS, M.D., F.R.C.P.(C), F.I.A.C.

AND

ROGER FORTIN, M.D., C.S.P.Q.

Department of Pathology and Cytodiagnostic Center, Saint-Sacrement Hospital and Laval University, Quebec, Canada

INTRODUCTION

Condylomata acuminata are hyperplastic, often vegetating and papillomatous lesions, caused by a virus (venereal wart agent) which may be identical to the causal agent of verruca vulgaris. The virus is most probably transmitted by venereal contact. The lesion is most frequently found in the vulva (Fig. 1), but may occur in the vagina (Fig. 2) and on the cervix (Fig. 3). The classical anatomopathologic description of this lesion is well known and need not be repeated here.

The diagnosis of vulvar lesions presents no particular difficulty. However, lesions in the vagina and on the cervix may be less obvious clinically but can be detected cytologically.

The first description of cells originating from condylomata acuminata was published in 1960 by Papanicolaou,⁹ who found "pronounced nuclear enlargements and in some cells marked hyperchromasia. All cells are, however, well differentiated superficial squamous cells with an abundant cytoplasmic supply." Papanicolaou warned that these cells may be confused with intraepithelial malignant lesions and pointed out the presence of perinuclear halos in some cells.

In 1961, Naib and Masukawa⁸ published a detailed description of their findings in 19 patients with condylomata acuminata involving the vagina or cervix. The cells were reported to resemble "large intermediate squamous cells. They appeared swollen

and edematous with a thick, pale, basophilic, poorly keratinized cytoplasm and poorly defined borders. The enlarged nuclei showed little variation in shape and usually had sharp, thick, regular nuclear borders. The chromatin pattern was bland and appeared edematous. The nucleolus was usually prominent, round, and regular. Within the nuclei, basophilic inclusion bodies varying in number, shape, and size were sometimes seen. Perinuclear halo, cytoplasmic degenerative vacuoles, binucleation, nuclear pycnosis and karyorrhexis were considered as secondary characteristics. The nuclear-cytoplasmic ratio was higher than that of normal intermediate cells but did not approach that of cells indicative of malignant change." Similar descriptions can be found in text-

FIG. 1. Colposcopic appearance of condylomata acuminata of the vulva.

Dr. Meisels is Professor of Pathology, Laval University and Head of the Cytodiagnostic Center, Saint-Sacrement Hospital, Quebec, Canada.

Dr. Fortin is Assistant Professor of Pathology, Laval University and Pathologist at the Saint-Sacrement Hospital, Quebec, Canada.

For reprints write to Alexander Meisels, M.D., Head of the Cytodiagnostic Service, Saint-Sacrement Hospital, 1050, Chemin Ste-Foy, Quebec, P.Q., Canada G1S 4L8.

The authors wish to thank Dr. Michel Roy for the colposcopic pictures.

FIG. 2. Colposcopic appearance of condylomatous lesions of the vagina.

books by Dupré-Froment⁴ and Tweedale.¹⁰ Koss⁷ cast doubt on this description and stated that cells shed from condylomata acuminata are "large squamous cells with slight to moderate nuclear enlargement and slight hyperchromasia. A perinuclear clear zone may be observed in some of these cells, making the differentiation from some types of dyskaryotic cells difficult, if not impossible. . . . Cytoplasmic or nuclear inclusions were not observed in our laboratory in this condition. The observations of Naib and Masukawa reporting "bland chromatin patterns and intranuclear basophilic inclusion bodies" presumably reflected a coexisting herpetic infection. In a recent illustration Bibbo and Wied² added that cellular samples from condylomata acuminata show "binucleated squames, parakeratotic cells and enlarged superficial, intermediate or parabasal cells with multinucleation, some cells with perinuclear halos, cytoplasmic vacuolization, coarse chromatin clumping, nuclear molding, prominent nucleoli, and cyanophilic inclusion-like bodies within the nuclei."

From the above descriptions, it appears that no clear cytologic pattern has as yet been identified, which would permit the unequivocal identification of condylomata acuminata from the cellular sample. A further element of confusion arises from the fact that condylomata acuminata may accompany dysplasia of the cervix and may even themselves display dysplastic changes which may evolve into carcinoma in situ and even invasive carcinoma of the cervix, as has been shown by Jagella and Stegner⁵ using histopathologic and cytophotometric techniques.

The present investigation was undertaken with the purpose of determining whether there is a characteristic cellular pattern, diagnostic for condyloma acuminata.

MATERIALS AND METHODS

The cytodiagnostic center at the St-Sacrement Hospital in Quebec City receives about 75,000 cellular samples from the female genital tract each

year. Since January 1975, all abnormal findings related to intraepithelial lesions of the cervix have been referred to colposcopy and small tissue samples have been obtained routinely from any visible lesion of cervix and vagina. We are now detecting condylomatous lesions of the vagina or the cervix at the rate of about 50 per month.

Simultaneously, we have been reviewing our material of about 7,000 mild and moderate dysplasias of the cervix and have found a number of lesions which were originally classified as mild dysplasias but later were proven to be condylomatous lesions.

Cellular Pattern of Condylomatous Lesions

The cell changes observed in our cases were quite typical. The smear was usually clean, with few or no inflammatory cells. The cell population consisted of intermediate and superficial cells only (all our patients were within the reproductive age groups). The cellular changes were observed on the portion of the vaginal-cervical-endocervical (V-C-E) smear corresponding to the site of the lesion.

There are essentially two characteristic changes: 1) Isolated cells, or more often small aggregates of cells show dyskeratotic changes with a dense yellowish or orangeophilic cytoplasm and small, dense nuclei of the size corresponding to the intermediate cell nucleus. Sometimes the dyskeratotic cells are much smaller than intermediate cells and contain a slightly irregular very small, dense nucleus.

FIG. 3. Colposcopic appearance of condylomatous lesions of the cervix.

FIGS. 4-11. These microphotographs were obtained from different V-C-E smears stained by the routine Papanicolaou technique, using a 40 $\times$ objective (original magnification 500 $\times$).

FIG. 4. Dyskeratotic area surrounded by several amphophilic balloon cells.

FIG. 5. Dyskeratotic area with amphophilic balloon cells.

FIG. 6. Several balloon cells, displaying amphophilia and dense, fuchsia-red staining.

FIG. 7. Characteristic appearance of balloon cells.

FIG. 8. Multinucleated balloon cell.

FIG. 9. Amphophilic balloon cells in a field of superficial squamous cells.

FIG. 10. Balloon cells with binucleation.

FIG. 11. A group of balloon cells displaying mild nuclear atypia.

2) The most typical change is the "balloon" cell: It is an intermediate cell, often quite enlarged, containing sometimes one, often two and occasionally several hyperchromatic slightly irregular nuclei. The chromatin is dense and may be coarsely granular. Nucleoli are either absent or inconspicuous. The nucleus is surrounded by an irregular clear area, which may vary in size from a small halo to a large empty space, occupying most of the cytoplasmic area. Peripheral to the perinuclear clearing, the cytoplasm is quite dense, almost glassy in appearance and shows a characteristic amphophilic staining.

Many cells will display two colors with the routine Papanicolaou technique, one is the bluish-green color normally seen in the cytoplasm of intermediate cells; the other is a deep, dense fuchsia-red hue quite different from the ordinary eosinophilia or orangeophilia. Sometimes this reddish stain is predominant, occasionally there are only a few reddish streaks (see color plate). No intranuclear or intracytoplasmic inclusions were observed in these cases.

The pattern of the smear may vary from a predominance of dyskeratotic changes with few balloon cells to scanty dyskeratotic changes with many balloon cells.

At colposcopy the lesions could be readily identified when they were situated on the vulva. In the vagina and on the cervix they were less papillomatous, appearing as white areas with moderate surface irregularities.

The lesions described were associated with histologic changes that were either consistent with the classical pattern of condyloma acuminatum or corresponded to a lesion similar in its internal structure but non-vegetating. This latter lesion will be described in greater detail in work now in progress.

DISCUSSION

The cellular pattern described is highly characteristic. Even when no lesion was seen at immediate colposcopy, condylomatous lesions appeared within two or three weeks following cytologic diagnosis. Most of the time, however, the lesion was already present at first colposcopy. The condylomatous cell pattern can be readily identified, once it has been recognized. The most obvious source of error lies in considering these patterns as a sign of cervical dysplasia. The literature contains several descriptions of balloon cells (Koilocytotic atypia^{3, 6} nearocarcinoma¹) without mention of dyskeratotic changes, which were considered as suggestive of dysplasia or early intraepithelial neoplasia.

Since condylomatous lesions are of viral origin and sexually transmitted, their frequency is high in young promiscuous females. Many of these lesions regress spontaneously. Whether they play a role in

the genesis of intraepithelial neoplasia and carcinoma of the cervix remains to be shown.

It seems clear however, that condylomatous lesions have been consistently diagnosed as mild dysplasia or Grade I intraepithelial neoplasia. Tissue sections may also easily be misinterpreted. Work in progress at the present time will attempt to identify the proper criteria for differential diagnosis on our biopsy material.

Condylomatous lesions of the cervix have a relatively high frequency in our screened population, similar to that of mild dysplasias. It may well be that once these lesions are properly identified, they will account for those "dysplasias" that regress spontaneously and do not fit in our present concept of development of intraepithelial and invasive neoplasia of the cervix.

SUMMARY

Condylomatous lesions, although readily diagnosed on the vulva, are often missed in the vagina and on the cervix by clinical examination alone. The lesions are, however, quite common and may be misdiagnosed as mild dysplasia by cytology, colposcopy and even tissue examination. Condylomatous lesions are presently diagnosed on cytologic evidence in nearly two per cent of asymptomatic patients screened in our program and followed-up by colposcopy and tissue examination, when indicated. The cytologic presentation of these lesions is quite characteristic. The main features are seen in squamous cells: enlargement, bi- or multinucleation, hyperchromasia, peri-nuclear clearing, amphophilia and dyskeratotic changes. Our present experience indicates that a large number of lesions previously classed as mild dysplasias actually represent various stages of condylomatous lesions. When these stages of viral changes are removed from the group of dysplasias, the remaining cases become of much greater significance as the early stages of evolution of carcinomata of the cervix.

REFERENCES

1. Ayre, J. E.: Role of the Halo Cell in Cervical Cancerigenesis. *Obstet. Gynec.* **15**: 481-491, 1960.
2. Bibbo, M. and Wied, G. L.: Identification of Inflammatory Reactions, Tissue Repair, Viral Infections and Microbiologic Classifications in Cytologic Specimens of the Female Reproductive Tract. Teaching Slide Sets in Cytology. Vol. 9. Tutorials of Cytology, Chicago, Ill. 1974.
3. De Girolami, E.: Perinuclear Halo versus Koilocytotic Atypia. *Obstet. Gynec.* **29**: 479-487, 1967.
4. Dupré-Froment, J.: *Cytologie Gynécologique* Flammarion, Paris, 1974. p. 76-77.
5. Jagella, H.P. and Stegner, H. E.: Zur Dignität der Condylomata acuminata. *Arch. Gynäk.* **216**: 119-132, 1974.

6. Koss, L. G. and Durfee, G. R.: Unusual patterns of squamous epithelium of uterine cervix; cytologic and pathologic study of Koilocytotic atypia. *Ann. N.Y. Acad. Sci.* **63**: 1235-1261, 1956.
7. Koss, L. G.: *Diagnostic Cytology and its Histopathologic Bases*. J.B. Lippincott Company, 2nd ed. p. 169, 1968.
8. Naib, Z. N. and Masukawa, N.: Identification of Condyloma Acuminata Cells in Routine Vaginal Smears. *Obstet. Gynec.* **13**: 735-738, 1961.
9. Papanicolaou, G. N.: *Atlas of Exfoliative Cytology*, Supplement 2, p AP 18-19, Harvard University Press, Cambridge, Mass. 1960.
10. Tweedale, D. N. and Dubilier, C. D.: *Cytopathology of Female Genital Tract Neoplasms*. Year Book Medical Publishers, Inc. Chicago, 1975, p. 76.

Cytology of Gynecologic Condyloma Acuminatum

ESKO PUROLA, M.D., M.I.A.C. AND EEVA SAVIA, M.D.

Laboratory of Pathology and Cytology, I-II Departments of Gynecology and Obstetrics, University Central Hospital of Helsinki, Helsinki, Finland

INTRODUCTION

The viral origin of condylomata acuminata, or genital warts, seems to be beyond doubt^{4, 13}, but the question as to whether these warts are caused by the same virus as are skin warts has not been definitely solved. On the other hand, it is quite clear that condylomata are mainly transmitted by sexual contact^{11, 12}. Although the lesions caused by viruses have received increasing attention during the last few years, surprisingly little has been written on the cytologic abnormalities of condylomata. The purpose of this investigation was to identify and describe the cells derived from genital condylomas as observed in vaginal and cervical smears.

MATERIALS AND METHODS

The present series consists of 192 condylomata. The material was collected from the files for 1970 and 1971 of the laboratories of cytology and pathology of Departments I and II of Obstetrics and Gynaecology, Helsinki University Central Hospital. In all cases in which a histo-pathologic diagnosis of condyloma had been made the specimens were reexamined. Ninety-four cases having the criteria of condyloma were included in the present material. The remaining 98 cases had not been biopsied but showed typical gross lesions clinically diagnosed as condyloma. The histologic diagnosis was chiefly based on the principles laid down by Woodruff and Peterson¹⁷, who stated that the most diagnostic features of condyloma are papillomatosis, elongation and thickening of the rete pegs, acanthosis, parakeratosis and cytoplasmic vacuolization. All squamous papillomas were included in the present material because the present authors doubt that etiologically different types of squamous papillary lesions occur in the female genital tract.

Most cases of condyloma occurred in young adult women, 79 per cent of the patients being from 16 to 25 years old. Fifty-eight patients (30%) were preg-

nant. Of these, 35 had been referred for legal abortion. The majority of the patients (151, or 78%) were para "0".

The topographical distribution of the condylomata is shown in Table I. Lesions confined only to the vulva or perineum were observed in 69 cases (36%, group A). Lesions exclusively found inside the vaginal orifice were seen in 55 cases (29%, group B): The vagina alone was involved in 13 of these cases, the cervix in 31, and both the cervix and the vagina in 11 cases. In 68 cases (35%, group C), condylomata occurred both inside and outside the vaginal orifice. Besides vulval condylomata, 36 of these patients had vaginal lesions, 16 had cervical lesions, and 16 had both cervical and vaginal warts. Thus the cervix was involved in 74 cases.

All of the present 192 cases were cytologically examined. The smears, obtained from the posterior vaginal fornix, the ectocervix and the endocervix, were spread across the same slide as proposed by Wied *et al.*¹⁶, and stained by Papanicolaou's original method.

RESULTS

Surface of the condylomatous epithelium

In order to get a proper grasp of the cytology of condyloma, the histologic study of specimens with particular attention to the surface of the epithelium of the vaginal and cervical condylomata was performed. With regard to the degree of surface differentiation the following three types were distinguished:

1. *Hyperkeratosis*. The surface consisted of a hyperkeratotic layer of anucleate squames. This type was often seen in the vulval warts, less often in the vaginal and cervical lesions.

2. *Parakeratosis*. The surface of the epithelium was keratinized, but the cells were nucleated or contained remnants of a nucleus. A type of the epithelial surface, which Koss⁸ calls "pseudoparakeratosis", was often seen. Irregular parakeratosis was a frequent finding: The nuclei of the keratinized cells were hyperchromatic, sometimes abnormal in shape and varying in size (Fig. 1). We use the term "dyskeratotic" for epithelium of this type.

3. *Intermediate*. A hyperplastic intermediate (prickle cell) layer extended to the surface. The surface of the epithelium thus consisted of intermediate squamous cells, which resembled the cells de-

Dr. Purola is Chief of the Laboratory of Cytology, Departments of Gynecology and Obstetrics, University Central Hospital, Helsinki, Finland.

Dr. Savia is a Resident at the II Department of Obstetrics and Gynaecology, University Central Hospital, Helsinki, Finland.

Correspondence: Dr. Esko Purola, HYKS Naistenklinikka, Haartmaninkatu 2, 00290 Helsinki 29, Finland.

TABLE I. Cytologic Findings and Location of Condylomas

Location of condylomas	Number of cases	(Per cent of total)	Cells suggestive of condyloma	
			Number of cases	(Per cent)
A. Only outside the vaginal orifice (vulva, perineum)	69	(36)	23	(33)
B. Only inside the vaginal orifice (vagina, cervix or both)	55	(29)	48	(87)
C. Both outside and inside the vaginal orifice	68	(35)	45	(66)
Total	192	(100)	116	(60)

FIG. 1. Parakeratotic epithelium covering a condylomatous papilla. The cells are smaller than the normal superficial cell. The nuclei are hyperchromatic, varying in size, and relatively large in relation to the cells. Some nuclei are surrounded by a halo formation. Histologic section of a cervical condyloma (van Gieson-haematoxylin, $\times 200$).

scribed below as "condylomatous" intermediate cells.

The superficial epithelial layer of most condylomata showed various degrees of differentiation.

Exfoliated cells

The following types of cells were considered to be exfoliated from a condylomatous epithelium:

1. *Dyskaryotic keratinized superficial cells.* We have called these cells dyskaryotic. Their cytoplasm is orangeophilic; the cells vary greatly in size and shape but are usually smaller than the normal superficial cell. An elongated, spindle-cell-like type is common (Figs. 5 and 6). The nuclei of these cells show atypia varying from slight hyperchromasia to marked dyskaryosis. Occasional cells with large

misshapen nuclei occur, which resemble keratinized carcinomatous cells. However, the most common type is a small, abnormally matured superficial cell with a round or elongated, strongly hyperchromatic nucleus, which is rather large in relation to the size of the cell (Figs. 1 and 2). The dyskaryotic cells often occur in sheets, in which the borders of the individual cells are indistinct; nuclear hyperchromasia, variation in size of the nuclei and yellow or orange staining of the cytoplasm are typical features (Figs. 5 and 6).

2. *"Condylomatous" intermediate cells.* These intermediate squamous cells vary in size and have poorly defined cell borders and a cyanophilic or, more often, unevenly stained polychromatic cytoplasm. Slight perinuclear halo formation and irregular vacuolization of the cytoplasm, other than perinuclear, can often be seen. The nuclei are enlarged having often accentuated nuclear membrane. The chromatin pattern is somewhat irregular, and the chromatin usually only slightly or not at all increased. This cell can sometimes be characterized as dyskaryotic on the basis of its nuclear structure; however, in many cases the nuclear changes are so slight that the term dyskaryotic is hardly justified. We have called this type of cell (Fig. 4), described above "condylomatous" for the reason that it was often encountered in the smears of condyloma patients and that it resembled the intermediate cells found in the surface of condylomata in histologic specimens.

3. *Koilocytotic cells* with a translucent area (a halo) surrounding a slightly dyskaryotic nucleus are a relatively frequent finding (Fig. 7). These cells are usually cyanophilic and resemble the above-mentioned intermediate type, but they may also be eosinophilic and more differentiated than the latter.

Intermediate forms between the types of cell described above were frequently observed. Slight halo formation was a common finding in dyskaryotic and condylomatous intermediate cells (Figs. 2 and 4). Only cells with a very distinct and large halo were classified as koilocytotic (Fig. 7). Moreover, the smears contained cells of certain other types presumably exfoliated from a condyloma: anuclear keratinized squames, regular parakeratotic cells with a very small pyknotic nucleus or remnants of a nucleus, and multinucleated giant cells. However, these types are relatively often encountered also in connection with lesions other than condyloma. Hence, they cannot be considered diagnostic or typical of condyloma acuminatum. Binucleation (Fig. 7) and various degenerative changes of the cytoplasm and the nucleus are common features in condylomatous cells of all types. Small cytoplasmic particles, which might be called inclusion bodies, occasionally occur. Intranuclear inclusion bodies of the kind typical of certain other virus infections were not observed in our material.

As may be seen in Table I, the smears of 116 (60.4%) of the 192 patients with condylomata con-

FIG. 2. Small dyskeratotic keratinized superficial cells in cervical smear. The nuclei vary in size but are large in relation to the cells, and markedly hyperchromatic. The cytoplasm stained orange-yellow. Note the resemblance with Figure 1. (Papanicolaou stain, $\times 200$).

FIG. 3. Epithelium of intermediate type covering a cervical condyloma. The surface consists of intermediate condylomatous squamous cells and koilocytotic cells. (van Gieson-haematoxylin, $\times 200$).

tained cells (dyskeratotic keratinized superficial cells, condylomatous intermediate cells, or koilocytotic cells) considered to be exfoliated from a condylomatous epithelium. In groups B and C, in which the condylomata were situated inside the vaginal orifice, cells of the above-mentioned types were found in 93/123 cases (76%). As regards the group with cervical condylomata, this finding was made in 64/74 cases (86%). In the group with clinical evidence of vulval condylomata alone (group A), cells suggestive of condyloma were found in 23/69 cases (33%).

The numbers and percentages of cases showing different types of cells suggestive of condyloma are shown in Table II. The occurrence of dyskeratotic cells in sheets is indicated in a separate column,

because this feature appeared to be particularly characteristic of condyloma.

Dysplasia associated with condyloma

If the term dysplasia is used for any disturbance of differentiation of the epithelium slighter than carcinoma in situ, as the Vienna Congress² proposed, a condylomatous epithelium is to be considered dysplastic. A diagnosis of condyloma then includes mild dysplasia without this being explicitly stated. However, in patients with condyloma the cervical or vaginal epithelium is often dysplastic without being truly condylomatous, since papillomatosis is lacking. In the present series, a diagnosis of dysplasia was not made if this type of epithelium was found in the same biopsy specimen as a condyloma. On the other hand, if dysplastic epithelium and condyloma were seen in different specimens, dysplasia was diagnosed.

The present series contained 94 cases in which

FIG. 4. Exfoliated condylomatous intermediate cell with indistinct cell borders and a hazy perinuclear halo formation. The cytoplasm is unevenly stained and shows both cyano- and eosinophilic components. The nucleus is enlarged, the chromatin pattern somewhat irregular. (Papanicolaou stain, $\times 800$).

FIG. 5. Thin parakeratotic surface layer of condylomatous epithelium exfoliating as a sheet. Histologic section of vaginal condyloma. (van Gieson-haematoxylin, $\times 400$).

FIG. 6. Parakeratotic sheet exfoliated from the surface of the epithelium of vaginal condyloma. The cell borders are indistinct, the cytoplasm stained orange-yellow, the nuclei are elongated. (Papanicolaou stain, $\times 200$).

FIG. 7. Koilocytotic cells in vaginal smear. The nucleus is surrounded by a large, sharply outlined halo. Typical binucleation is seen in two cells. (Papanicolaou stain, $\times 400$).

colposcopy had been performed and vaginal or cervical biopsy specimens were available. Mild or moderate dysplasia of the cervical epithelium was observed in 22 cases, mild dysplasia of the vaginal epithelium in seven, and severe dysplasia of the cervical epithelium in two cases. Some structural features typical of condyloma, such as hyperplasia, elongated rete pegs, parakeratosis, hyperkeratosis and vacuolization, were seen in all of the above-mentioned cases of dysplasia associated with condyloma. The cytologic picture in those cases showing mild dysplasia of the vaginal or cervical epithelium did not differ qualitatively from that seen in connection with condyloma without dysplasia. Greater cytologic variety was seen in the epithelial atypia classified as severe dysplasia; dyskaryotic cells of different types, including parabasal cells, were seen in the smear.

DISCUSSION

In the relevant literature, squamous papillomas have usually been classified into two different types: "single" or "true papilloma assumed to be an, at least to some extent, potentially malignant true neoplasm of unknown etiology and condyloma acuminatum, described as a hyperplastic epithelial lesion presumably caused by a virus.^{3, 4} Most writers on this subject have been unable to present histologic criteria distinct enough for a differentiation between true papilloma and condyloma acuminatum. Koss⁸ maintains the above classification, but he states at the same time that solitary papilloma is structurally and cytologically similar to a condyloma acuminatum. Goforth⁶ and Woodroff and Peterson¹⁷ stated that

FIG. 8. Dysplasia of the cervical epithelium showing the structural features typical of condyloma: hyperplasia, deep rete pegs, marked vacuolization of the cells and keratinization of individual cells in an acanthotic layer. Parakeratosis was more conspicuous in some other areas of the same specimen. The patient had vulval condylomata and cervical leukoplakia measuring 2×3 cm, of which a histologic specimen was taken. An abundance of dyskaryotic keratinized superficial cells, condylomatous intermediate cells and halo cells were seen in cervical smear. (van Gieson-haematoxylin, $\times 100$).

TABLE II. The Occurrence of Various Types of Cells Suggestive of Condyloma in a Series of 192 Patients with Gynaecologic Condylomata

Cell type	Number of patients	Per cent
Dyskaryotic keratinized superficial cells	112	58
Dyskaryotic keratinized superficial cells in sheets	74	39
Dyskaryotic intermediate cells of condyloma type	97	51
Koilocytotic cells	58	30

true papilloma can be distinguished from condyloma acuminatum by the presence of hyperkeratosis and, often, by a granular layer, more prominent papillomatosis, minimal or absent vacuolization and a lesser degree of acanthosis. However, these criteria have not been universally accepted.^{7, 8} We were also unable to classify the squamous cell papillomas into two distinct histologic types. According to our experience, structural features considered typical of condyloma or true papilloma, respectively, are often seen in one and the same lesion. To diagnose a squamous papillary lesion as condyloma because similar lesions are present in the vulva or vagina and as a papilloma when the lesion is situated in the cervix does not seem justified. The viral etiology of condyloma acuminatum is not disputed. In our opinion there is no ground for assuming that the etiology of a solitary lesion is different.

The true incidence of condyloma is not known. Many patients with genital warts do not seek medical aid, because the disease is innocent and causes few symptoms. Cervical condylomata are evidently relatively rare. Pitkin and Kent,¹⁴ who surveyed the literature in 1963, found 173 cases and reported 14 own cases. The proportion of cervical lesions in the present series appears high, but the material is selected; the outpatient department of our hospital is visited by patients referred for colposcopy and biopsy on account of positive cytologic findings in smears taken at mass screening. This explains the high frequency of cervical condylomata. On the other hand, a considerable proportion of vulval warts have certainly escaped notice, because the lesion was not mentioned in the referring letter.

Abnormalities in cytologic smears due to condyloma have seldom been mentioned in the literature. Koss⁸ made the following statement about the cytology of these lesions: "Occasionally, condylomata shed large squamous cells with slight to moderate nuclear enlargement and slight hyperchromasia. A perinuclear clear zone may be observed in some of these cells making the differentiation from some types of dyskaryotic cells difficult, if not impossible." Ayre,¹ also, drew attention to the occurrence of koilocytotic cells. He assumed that a premalignant viral manifestation was involved. In a filmstrip presentation of exfoliative cytology, Stafford¹⁵ established that slight nuclear hyperactivity and koilocytosis are findings typical of condyloma. Naib and Masukawa⁹ reported that 13 out of 19 patients studied had typical cells in their smears considered by the authors to be shed from condylomatous lesions. According to the description, the cells they regarded as typical were very like those described in the foregoing as "intermediate condylomatous cells". Naib and Masukawa found intranuclear cyanophilic inclusion bodies in the cells in question. However, Koss⁸ presumed that this finding reflected a coexisting herpetic infection.

The frequent occurrence of mild dysplastic epithelial atypia in association with condyloma in the present material is noteworthy. Attention was particularly drawn to the fact that structural features typical of the condylomatous epithelium were common in these dysplasias. A borderline lesion of the type for which Koss used the term "koilocytotic or warty atypia" was encountered in many biopsy specimens. Although direct evidence is lacking, it seems probable that these abnormalities are changes of the squamous epithelium caused by condyloma virus. However, the possibility that these dysplasias are true precancerous lesions associated with condylomata cannot be ruled out except by many years of follow-up without treatment. For this reason, the present patients are regularly seen and the course of the lesions is attentively followed. The possibility of viral agents playing a part in the genesis of cervical neoplasia has been suggested by numerous studies, and the herpes II virus has repeatedly been implicated. As pointed out by Novak and Woodruff,¹⁰ condyloma acuminatum may assume additional significance in this connection. Since we started closely investigating the histology and cytology of condyloma, our attention was drawn to the fact that morphologic features suggesting condyloma are often seen in both cytologic and histologic specimens of cervical and, in particular, vaginal dysplasias not associated with condyloma and also in highly differentiated squamous carcinomas. Although it is quite clear that morphologic resemblance is not necessarily a sign of etiologic identity, we consider it possible that condyloma infection may play a part in the genesis of these lesions.

The present results seem to indicate that condyloma virus may cause lesions of the vaginal and cervical epithelium of the following types:

1. Macroscopically visible papillomatosis or deep folding of the epithelium, called condyloma, single or multiple.
2. A condyloma not discernible macroscopically but recognized by the aid of the colposcope.
3. A condyloma not demonstrable by colposcopy, although microscopic examination of a biopsy specimen reveals a typical papillary epithelium. The usual colposcopic finding in these cases is leukoplakia, sometimes mosaic or ground.
4. Mild, moderate or severe dysplasia showing microscopical structural features typical of condyloma, although papillary formation are lacking.
5. A lesion too small to be clinically observable; it is revealed only by the presence in cytologic smears of occasional cells indicating condyloma infection. The fact that such cells were found in one-third of the smears in group A of the present material suggests

that patients with clinically diagnosed condylomata only in the vulva often have slight condylomatous lesions also in the epithelium of the vagina and/or cervix.

SUMMARY

The morphology of certain cell types often encountered in vaginal and cervical smears of condyloma patients are described. These cells, considered to be exfoliated from epithelium affected by condyloma virus, were found in 60 per cent of the smears obtained from 192 women with condylomata acuminata. Dysplasia of the cervical and vaginal epithelium is frequently associated with condyloma. Structural features typical of the condylomatous epithelium were common in these dysplasias. These lesions are probably also due to infection by condyloma virus, yet their significance is unknown and the possibility of a precancerous status cannot be ruled out except by a very long term follow-up study which is in progress. It may be assumed that this virus may affect the cervical and vaginal epithelium without any typical papillary condylomatous lesions resulting. Even in the absence of typical condylomatous papillary formations, the cytologic findings may reveal condyloma infection.

REFERENCES

1. Ayre, J. E.: Role of the Halo Cell in Cervical Carcinogenesis. *Obstet. Gynec.* **15**: 481-491, 1960.
2. Bettinger, H. F. and Reagan, J. W.: Proceedings of the international terminology for lesions of the uterine cervix. In Wied, G. L., Ed.: *Proceedings of the First International Congress on Exfoliative Cytology*. Philadelphia, J. B. Lippincott Co., 1962.
3. Daugherty, C. M.: *Surgical Pathology of Gynecologic Disease*. Hoeber Medical Division, Harper & Row Publishers. New York, Evanston and London 1968, p. 130.
4. Dunn, A. E. N. and Olgilvie, M. M.: Intranuclear virus particles in human genital wart tissue; observations on the ultrastructure of the epidermal layer. *J. Ultrastruct. Res.* **22**: 282-295, 1968.
5. Fluhman, C. F.: *The Cervix Uteri and Its Diseases*. W. B. Saunders Company, Philadelphia-London 1961, p. 240.
6. Goforth, J. L.: Polyps and papillomas of the cervix uteri. *Texas St. J. of Med.* **49**: 81-85, 1953.
7. Kazal, L. H. and Long, J. P.: Squamous cell papillomas of the uterine cervix. *Cancer* **11**: 1049-1059, 1958.
8. Koss, L. G.: *Diagnostic Cytology and Its Histopathologic Bases*. 2nd Edit., p. B. Lippincott Co., Philadelphia, 1968, p. 152, 169, 206.
9. Naib, Z. M. and Masukawa, N.: Identification of condyloma acuminata cells in routine vaginal smears. *Obstet. Gynec.* **18**: 735-738, 1961.
10. Novak, E. R. and Woodruff, J. D.: *Novak's Gynecologic and Obstetric Pathology*. 7th Edit. W. B. Saunders Company. Philadelphia-London-Toronto 1974, p. 90.
11. Oriel, J. D.: Natural history of genital warts. *Brit. J. Vener. Dis.* **47**: 1-13, 1971.
12. Oriel, J. D.: Anal warts and anal coitus. *Brit. J. Vener. Dis.* **47**: 373-376, 1971.
13. Oriel, J. D. and Almeida, J. D.: Demonstration of virus particles in human genital warts. *Brit. J. Vener. Dis.* **46**: 37-40, 1970.
14. Pitkin, R. M. and Kent, T. H.: Papillary squamous lesions of the uterine cervix. *Amer. J. Obstet. Gynec.* **85**: 440-446, 1963.
15. Stafford, M. W.: Exfoliative cytology. A Filmstrip presentation. Unit I. W. B. Saunders Co., Philadelphia-London-Toronto, 1972.
16. Wied, G. L. and Bahr, G. F.: Vaginal, cervical and endocervical cytologic smears on a single slide. *Obstet. Gynec.* **14**: 362-367, 1959.
17. Woodruff, J. D. and Peterson, W. F.: Condylomata acuminata of the cervix. *Amer. J. Obstet. Gynec.* **75**: 1353-1361, 1958.

References

- Meisels A, Fortin R: Condylomatous lesions of the cervix and vagina. 1. Cytologic patterns. *Acta Cytol* 1976;20:505–509.
- Purola E, Savia E: Cytology of gynecologic condyloma acuminatum. *Acta Cytol* 1977;21:26–31.
- Joura EA, Pils S: Vaccines against human papillomavirus infections: protection against cancer, genital warts or both? *Clin Microbiol Infect* 2016;22:(suppl 5):S125–S127.
- Syrjänen KJ: Annual disease burden due to human papillomavirus (HPV) 16 and 18 infections in Finland. *Scand J Infect Dis* 2009;108:2–32.
- Koss LG: Comments on the previous milestones article. *ASIP Bull* 2003;6:13–15.
- Vesterinen E: Papa-kokeen kertomaa (“Story of the PAP test”). Helsinki, Edita Prima, 2004, pp 58–63.
- Lynch RG: Condylomata, HPV and cervical cancer. *ASIP Bull* 2003;6:10–11.
- Syvertson JT, Dascomb HE, Wells EB, Kooman J, Berry GP: The virus-induced papilloma to carcinoma sequence. Carcinomas in the natural host, the cottontail rabbit. *Cancer Res* 1950;10:440–444.
- Bärfverstedt B: Condylomata acuminata past and present. *Acta Derm Venereol (Stockh)* 1967;47:376–381.
- Syrjänen K, Syrjänen S: Overview of papillomavirus research; in Syrjänen K (ed): *Papillomavirus Infections in Human Pathology*. New York, J. Wiley and Sons, 2000, pp 1–10.
- Syrjänen S, Syrjänen K: The history of papillomavirus research. *Cent Eur J Pub Health* 2008;16:S7–S13.
- zur Hausen H: Papillomaviruses in the causation of human cancers – a brief historical account. *Virology* 2009;384:260–265.
- Pyrhönen S, Penttinen K: Wart virus antibodies and the prognosis of wart disease. *Lancet* 1972;2:1330–1332.
- Pyrhönen S, Johansson E: Regression of warts. An immunological study. *Lancet* 1975;1:592–596.
- Pyrhönen S: Human wart virus antibodies in patients with genital and skin warts. *Acta Derm Venereol Stockh* 1978;58:427–432.
- Pyrhönen S, Neuvonen E: The occurrence of human wart virus antibodies in dogs, pigs, and cattle. *Arch Virol* 1978;57:297–305.
- zur Hausen H, Meinhof W, Scheiber W, Bornkamm GW: Attempts to detect virus-specific DNA in human tumors. I. Nucleic acid hybridizations with complementary RNA of human wart virus. *Int J Cancer* 1974;13:650–656.
- zur Hausen H, Gissmann L, Steiner W, Dippold W, Dregger I: Human papilloma virus and cancer. *Bibl Haematol* 1975;43:569–571.
- zur Hausen H: Condylomata acuminata and human genital cancer. *Cancer Res* 1976;36:794.
- zur Hausen H: Human papillomaviruses and their possible role in squamous cell carcinomas. *Curr Top Microbiol Immunol* 1977;78:1–30.
- Gissmann L, zur Hausen H: Human papilloma virus DNA: physical mapping and genetic heterogeneity. *Proc Natl Acad Sci USA* 1976;73:1310–1313.
- Gissmann L, Pfister H, zur Hausen H: Human papilloma viruses (HPV): characterization of four different isolates. *Virology* 1977;76:569–580.
- Orth G, Favre M, Croissant O: Characterization of a new type of human papillomavirus that causes skin warts. *J Virol* 1977;24:108–120.
- Pfister H, zur Hausen H: Seroepidemiological studies of human papilloma virus (HPV 1) infections. *Int J Cancer* 1978;21:161–165.
- Syrjänen KJ: Light source. *CytoPaths* 2006;23:5–6.
- Syrjänen KJ: Morphologic survey of the condylomatous lesions in dysplastic and neoplastic epithelium of the uterine cervix. *Arch Gynaecol* 1979;227:153–161.
- Syrjänen KJ: Current views on the condylomatous lesions in uterine cervix and their possible relationship to cervical squamous cell carcinoma. *Obstet Gynecol Surv* 1980;35:685–694.
- Syrjänen KJ, Heinonen U-M, Kauraniemi T: Cytological evidence of the association of condylomatous lesions with the dysplastic and neoplastic changes in uterine cervix. *Acta Cytol* 1981;25:17–22.
- Della Torre G, Pilotti S, De Palo G, Rilke F: Viral particles in cervical condylomatous lesions. *Tumori* 1978;64:549–553.
- Hills E, Lavery CR: Electron microscope detection of papillomavirus particles in selected koilocytotic cells in routine cervical smears. *Acta Cytol* 1979;23:53–56.
- Dunn AEG, Ogilvie MM: Intracellular virus particles in human genital wart tissue observations on the ultrastructure of the epidermal layer. *J Ultrastr Res* 1968;22:282–295.
- Almeida JD, Oriel JD, Stannard LM: Characterization of the virus found in human genital warts. *Microbios* 1969;3:225–232.
- Strauss MJ, Shaw EW, Bunting HL, Melnick J: “Crystalline” virus-like particles from skin papillomas characterized by intranuclear inclusion bodies. *Proc Soc Exp Biol Med* 1949;72:46–50.
- Syrjänen KJ: Histological and cytological evidence of a condylomatous lesion in association with an invasive carcinoma of uterine cervix. *Arch Geschwulstforsch* 1979;49:436–443.
- Orth G, Jablonska S, Favre M, Croissant O, Jarzabek-Chorzelska M, Rceza G: Characterization of two types of human papillomaviruses in lesions of epidermodysplasia verruciformis. *Proc Natl Acad Sci USA* 1978;75:1537–1541.
- Quick CA, Faras A, Krzysek R: The etiology of laryngeal papillomatosis. *Laryngoscope* 1978;88:1789–1795.
- Quick CA, Foucar E, Deliver LP: Frequency and significance of epithelial atypia in laryngeal papillomatosis. *Laryngoscope* 1979;89:550–560.
- Quick CA, Watts SL, Krzysek RA, Faras AJ: Relationship between condylomata and laryngeal papillomata. Clinical and molecular virological evidence. *Ann Otol Rhinol Laryngol* 1980;89:467–471.
- Syrjänen KJ, Syrjänen SM: Histological evidence for the presence of condylomatous epithelial lesions in association with laryngeal squamous cell carcinoma. *ORL* 1981;43:181–194.
- Syrjänen KJ, Syrjänen SM, Pyrhönen S: Human papilloma virus (HPV) antigens in lesions of laryngeal squamous cell carcinomas. *ORL* 1982;44:323–334.
- Gama RR, Carvalho AL, Longatto Filho A, Scorsato AP, Mendoza López RV, Rautava J, Syrjänen S, Syrjänen K: Detection of human papillomavirus (HPV) in laryngeal squamous cell carcinoma (LSCC): systematic review and meta-analysis. *Laryngoscope* 2016;126:885–893.
- Syrjänen KJ: Condylomatous changes in neoplastic bronchial epithelium. Report of a case. *Respiration* 1979;38:299–304.
- Syrjänen KJ: Bronchial squamous cell carcinomas associated with epithelial changes identical to condylomatous lesions of the uterine cervix. *Lung* 1980;158:131–142.
- Syrjänen KJ: Detection of human papillomavirus (HPV) in lung cancer: systematic review and meta-analysis. *Anticancer Res* 2012;32:3235–3250.
- Law MF, Lancaster WD, Howley PM: Conserved polynucleotide sequences among the genomes of papilloma viruses. *J Virol* 1978;32:199–211.
- Coggin JR, zur Hausen H: Workshop on papillomaviruses and cancer. *Cancer Res* 1979;39:545–546.
- de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H: Classification of papillomaviruses. *Virology* 2004;324:17–27.
- de Villiers EM: Cross-roads in the classification of papillomaviruses. *Virology* 2013;445:2–10.
- Gissmann L, zur Hausen H: Partial characterization of viral DNA from human genital warts (condylomata acuminata). *Int J Cancer* 1980;25:605–609.
- de Villiers EM, Gissmann L, zur Hausen H: Molecular cloning of viral DNA from human genital warts. *J Virol* 1981;40:932–935.
- Gissmann L, Diehl V, Schultz Coulon HJ, zur Hausen H: Molecular cloning and characterization of human papilloma virus DNA derived from a laryngeal papilloma. *J Virol* 1982;44:393–400.

- 52 Dürst M, Gissmann L, Ikenberg H, zur Hausen H: A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci USA* 1983;80:3812–3815.
- 53 Syrjänen K, de Villiers EM, Väyrynen M, Mäntyjärvi R, Parkkinen S, Castren O: Cervical papillomavirus infection progressing to invasive cancer in less than three years. *Lancet* 1985;325:510–511.
- 54 Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H: A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *EMBO J* 1984;3:1151–1157.
- 55 Lorincz AT, Lancaster WD, Temple GF: Cloning and characterization of the DNA of a new human papillomavirus associated with dysplasia of the uterine cervix. *J Virol* 1986;58:225–229.
- 56 Beaudenon S, Kremsdorf D, Croissant O, Jablonska S, Wain Hobson S, Orth G: A novel type of human papillomavirus associated with genital neoplasias. *Nature* 1986;321:246–249.
- 57 Syrjänen K, Väyrynen M, Castren O, Yliskoski M, Mäntyjärvi R, Pyrhönen S, Saarikoski S: Sexual behaviour of the females with human papillomavirus (HPV) lesions in the uterine cervix. *Brit J Vener Dis* 1984;60:243–248.
- 58 Syrjänen KJ, Pyrhönen S, Aukee S, Koskela E: Squamous cell papilloma of the oesophagus: a tumour probably caused by human papilloma virus (HPV). *Diagn Histopathol* 1982;5:291–296.
- 59 Syrjänen KJ: Histological changes identical to those of condylomatous lesions found in esophageal squamous cell carcinomas. *Arch Geschwulstforsch* 1982;52:283–292.
- 60 Syrjänen KJ, Pyrhönen S, Syrjänen SM: Evidence suggesting human papillomavirus (HPV) etiology for the squamous cell papilloma of the paranasal sinus. *Arch Geschwulstforsch* 1983;53:77–82.
- 61 Syrjänen KJ: Geographic origin is a significant determinant of human papillomavirus (HPV) prevalence in esophageal squamous cell carcinoma: systematic review and meta-analysis. *Scand J Infect Dis* 2013;45:1–18.
- 62 Syrjänen K, Syrjänen S: Detection of human papillomavirus (HPV) in sinonasal papillomas: systematic review and meta-analysis. *Laryngoscope* 2013;123:181–192.
- 63 Jenson AB, Lancaster WD, Hartmann DP, Shaffer EL Jr: Frequency and distribution of papillomavirus structural antigens in verrucae, multiple papillomas, and condylomata of the oral cavity. *Am J Pathol* 1982;107:212–218.
- 64 Syrjänen KJ, Pyrhönen S, Syrjänen SM, Lamberg MA: Immunohistochemical demonstration of human papilloma virus (HPV) antigens in oral squamous cell lesions. *Brit J Oral Surg* 1983;21:147–153.
- 65 Syrjänen K, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J: Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. *Int J Oral Surg* 1984;12:418–424.
- 66 Syrjänen S: Human papillomavirus (HPV) in head and neck cancer. *J Clin Virol* 2005;32(suppl 1):S59–S66.
- 67 Syrjänen S: Human papillomaviruses in head and neck carcinomas. *N Engl J Med* 2007;356:1993–1995.
- 68 Syrjänen S, Rautava J, Syrjänen K: HPV in head and neck cancer – 30 years of history. *Recent Results Cancer Res* 2017;206:3–25.
- 69 Kreider JW, Howett MK, Wolfe SA, Bartlett GL, Zaino RJ, Sedlacek T, Mortel R: Morphological transformation in vivo of human uterine cervix with papillomavirus from condylomata acuminata. *Nature* 1985;317:639–641.
- 70 Beckman AM, Myerson D, Daling JR, Kiviat NB, Fenoglio CM, McDougall JK: Detection and localization of human papillomavirus DNA in human genital condylomas by in situ hybridization with biotinylated probes. *J Med Virol* 1985;16:265–273.
- 71 Gupta J, Gendelman HE, Naghashfar Z, Gupta P, Rosenshein N, Sawada E, Woodruff JD, Shah K: Specific identification of human papillomavirus type in cervical smears and paraffin sections by in situ hybridization with radioactive probes: a preliminary communication. *Int J Gynecol Pathol* 1985;4:211–218.
- 72 Syrjänen S, Syrjänen K: An improved in situ DNA hybridization protocol for detection of human papillomavirus (HPV) DNA sequences in paraffin-embedded biopsies. *J Virol Meth* 1986;14:293–304.
- 73 Syrjänen K, Gissmann L, Koss LG (eds): *Papillomaviruses and Human Disease*. Berlin, Springer, 1987, pp 1–518.
- 74 Yasumoto S, Burkhardt AL, Doniger J, DiPaolo JA: Human papillomavirus type 16 DNA induced malignant transformation of NIH 3T3 cells. *J Virol* 1986;57:572–577.
- 75 Pirisi L, Yasumoto S, Feller M, Doniger J, DiPaolo JA: Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. *J Virol* 1987;61:1061–1066.
- 76 Dürst M, Dzarlieva Petrusevska RT, Boukamp P, Fusenig NE, Gissmann L: Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. *Oncogene* 1987;1:251–256.
- 77 McCance DJ, Kopan R, Fuchs E, Laimins LA: Human papillomavirus type 16 alters human epithelial cell differentiation in vitro. *Proc Natl Acad Sci USA* 1988;85:7169–7173.
- 78 Dyson N, Howley PM, Munger K, Harlow E: The human papilloma virus 16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989;243:934–937.
- 79 Werness BA, Levine AJ, Howley PM: Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 1990;248:76–79.
- 80 Kirnbauer R, Booy F, Cheng N, Lowy DR, Schiller JT: Papillomavirus L1 major capsid protein self assembles into virus like particles that are highly immunogenic. *Proc Natl Acad Sci USA* 1992;89:12180–12184.
- 81 Ayre JE: The vaginal smear: “precancer” cell studies using a modified technique. *Am J Obstet Gynecol* 1949;58:1205–1219.
- 82 Koss LG, Durfee GR: Unusual patterns of squamous epithelium of the uterine cervix: cytologic and pathologic study of koilocytotic atypia. *Ann NY Acad Sci* 1956;63:1245–1261.