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Cervical precancer and cancer, past, present and future

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Summary

This review is a short summary of the very long history of invasive and in situ carcinomas of the cervix.

The items considered in this paper are the etiology of cervical cancer by a sexually transmitted agent proposed about 150 years ago by Domenico Rigoni Stern, the birth of radical surgery for the treatment of cervical invasive carcinoma with the Wertheim operation in 1898, radium therapy and chemotherapy, cytological diagnosis, the birth of colposcopy, microcolposcopy, the definition of carcinoma in situ, dysplasia and microcarcinoma, the birth of the International Federation for Cervical Pathology and Colposcopy, condylomatosis lesions of the cervix and some HPVs as agents of cervical pre-cancer and cancer, and finally the concept of vaccination against oncogenic HPV types. All these constitute an integral part of common medical practice.

Key words: Cervical cancer; in situ carcinoma; History.

The historical concept of a sexually transmitted agent in the etiology of cervical cancer

In 1842 an Italian physician named Domenico Rigoni Stern reported in a Venetian journal "Giornale per servire ai progressi della patologia e della terapeutica", that from a study of the General Registry Office of Verona, women who died of cancer of the uterus were all married whereas cancer of the uterus was absent in virgins (quoted by Cislaghi [1]). This observation led this author to the idea that cancer of the cervix, and it is worth nothing that in the 19th century cancer of the uterus was predominantly cancer of the cervix, a sexual factor might be important. At present, the role of a sexually transmitted agent in the etiology of squamous cell carcinoma of the uterine cervix and its precursors is well known.

The birth of radical therapy for invasive cervical carcinoma

In 1898 Wertheim introduced abdominal radical hysterectomy with removal of the adjacent medial portions of parametria and the upper part of the vagina.

In those days, radical surgery for cervical cancer was fraught with risk. With the lack of general education and crude clinical diagnostic methods, presentation with late stage disease and anemia was common. Operability rate at best, was never more than 50%.

The Wertheim operation was a heroic and dramatic operation. It was performed under anaesthesia with chroloform dripped through a gauze mask. There was no treatment for a possible infection since sulphamides and antibiotics still had to be discovered. There was no blood transfusion possibility as the first haematic antigens were identified only at the beginning of the next century. There was no intravenous therapy. There was no efficacious therapy in case of shock. Should a shock have occurred, Wertheim could only have the patient wrapped in warm blankets, have small cognac enemas administered, plus hypodermoclysis. Mortality in the first cases was reported as 40%, but in subsequent published material, mortality rate was reduced to 18% [2].

Pelvic lymphadenectomy was not part of the Wertheim procedure, pelvic lymphadenectomy was added to abdominal hysterectomy according to Wertheim, by Meigs only in 1945 [3].

Around the same time of Wertheim, Schauta introduced vaginal radical hysterectomy [4] and in 1908 [5] he reported a significant reduction in the mortality rate utilizing this approach. Amreich [6] elaborated and improved on Schauta's original technique. However following Meigs' publication [3], the popularity of the radical vaginal hysterectomy declined.

Schauta's operation was reproposed recently by Dargent [7] in early stage cervical cancer, when a presurgical pelviscopy had shown negative lymph nodes.

In the same year of Wertheim, Marie Sklodowska Curie and Pierre Curie discovered radium [8]. In 1913, Cheron and Rubens-Duval [9] first reported encouraging results with radium in the treatment of 152 patients with cervical carcinomas.

Besides surgery, radium therapy and external radiation therapy, also chemotherapy is an arm of the armamentarium against cervical cancer. The date of birth of chemotherapy is 1943. An English ship loaded with mustard gas foundered in the seaport of Bari. Physicians noted severe myelodepression in the shipwrecked persons. A task force (one of the members was Karnofsky) was established by the director of the Memorial Hospital of New York with the aim of studying the effects of yperite compounds in experimental animals. As a consequence of these studies the first chemotherapeutic drug, mechloretamine, against cancer was developed.

In cervical cancer, besides the treatment in advanced or relapsed cases, chemotherapy appears useful as a neoadjuvant treatment before surgery in no early stages of disease [10] and in combination with radiotherapy in locally advanced cervical cancer. In 1999, after phase III studies on concomitant chemotherapy-radiotherapy demonstrating improved survival in locally advanced cervical cancer [11] a clinical announcement by the National Cancer Institute of Bethesda was the following: "strong consideration should be given to the incorporation of concurrent cisplatin-based chemotherapy with radiation therapy in women who require radiation therapy for treatment of cervical cancer" (quoted by Thomas) [11].

The birth of colposcopy

In 1921 Hans Hinselmann while deputy professor at Hamburg University commenced his studies in better observing the portio. With the help of Leitz technicians, he devised the first working binocular colposcope. In 1925 Hinselmann published the first paper on colposcopy [12] and in 1933 the book "Einfuhrung in die kolposcopie" [13].

For a long time, colposcopy was restricted exclusively to Germany and thereafter to Central and Southern Europe. With the exception of Argentina and Brazil, in which colposcopy was introduced in 1932 and 1934 by visiting professors Jakob and De Morales, respectively (reported by De Palo, Chanen and Dexeus) [14], it was not fully accepted elsewhere due to difficulties in training and understanding the original German nomenclature, for the rise of Nazism in Germany with the threat of the Second World War, that created barriers between Germany and the rest of the world.

For example, for many years in the USA colposcopy was ignored. As reported by Di Saia and Creasman [15], initial efforts were made in the early 1930's to introduce colposcopy in the USA, but the method was ignored. The interest was renewed in the middle of the 1950's although acceptance was low because of the competition of the Pap test. Only after 1964, with the foundation of a specific society, did colposcopy gain some popularity and become recognized as an adjunctive technique to cytology.

Colposcopy is now universally accepted as a means of studying physiology and pathology of the lower genital tract and as a means for diagnosis not only of an early invasive or precancerous lesion but also, as a means of determining the site, size and extent of that lesion. Colposcopy dictates the necessity for a confirmed histological diagnosis by directed target or cone biopsies.

As reported in the Walton report on cervical cancer screening "Colposcopic examination should not be regarded as a screening technique, but as an important diagnostic tool for the localization and assessment of premalignant disease and early invasive carcinoma of the cervix in women with abnormal cervical smears" [16]. Therefore it is not only a predictor of an underlying histologic diagnosis in those reported to have an abnormal pap smear, but it is also of great value in selecting what might be the most appropriate treatment for that specific lesion.

Microcolposcopy

In 1981 Hamou [17] introduced the microcolpohysteroscope for examination of the cervix and endocervical canal. It provides a panoramic and contact microscopic observation of stained cells in vivo at high magnification. Contrast of cellular elements is obtained by coloring the tissue with a vital stain, e.g. Water-

man's blue ink which will selectively stain squamous epithelium or squamous metaplastic tissue in its various stages of maturation, leaving the cylindrical cells unstained so that the boundary of the upper limit of normal or pathologic epithelium will be clearly defined within endocervical canal [18].

Besides the "in vivo" study of cervical squamous epithelial cells, the practical indication of microcolposcopy is to visualize the squamo-columnar junction and to define the upper limit of an abnormal transformation zone or CIN extending into the canal, prior to embarking on a cone biopsy [19].

The birth of cytological diagnosis

In about the year 1924, George Papanicolaou, an investigator at Cornell University, interested in the cell changes during the menstrual cycle, made an incidental observation that cancer cells derived from the uterine cervix may be observed in human vaginal smears when collected by a glass pipette. He presented these observations in May 1928 at the Third Race Betterment Conference (reported by Koss) [20]. At the same time, Babes, a Rumanian pathologist, wrote on the possibility of a diagnosis of cervical cancer by cervical smears obtained with a bacteriologic loop and published a paper entitled "Diagnostique du cancer uterin par les frottis" [21].

Afterwards Papanicolaou, from the vaginal pool smears provided by the gynecologist Traut, identified cancer cells in a number of patients with malignant tumors of the uterine cervix, some not suspected clinically. In 1943 Papanicolaou and Traut published the book "Diagnosis of the uterine cancer by the vaginal smear" [22].

In 1947 (23) Ayre documented that a sample obtained directly from the uterine cervix by a wooden spatula was more efficient and easier to examine than a vaginal smear; after many years a further device was developed for sampling from the endocervical canal [24].

With the identification of cancerous and precancerous changes in cytologic samples, the so-called pap test was considered as the ultimate tool in cancer detection and prevention.

Carcinoma in situ and dysplasia

The concept and the term carcinoma in situ was introduced in 1932 by Broders [25] and the term dysplasia by Reagan *et al.* in 1953 [26]. The International Committee on Histological Definitions specified the two terms in 1962 [27], dysplasia being defined as "all disturbances of differentiation of the squamous epithelium of lesser degree than carcinoma in situ". Koss in 1963 reported that regardless of morphologic appearance, all precancerous intraepithelial abnormalities of the uterine cervix are capable of progression to invasive cancer, albeit with a lower frequency for "mild dysplasia" and a higher frequency for "severe dysplasia" [28]. In 1967 Richart suggested the term cervical intraepithelial neoplasia.

Cervical intraepithelial neoplasia encompassed all grades of dysplasia and carcinoma in situ, with dysplasia and carcinoma in situ constituting a histological continuum [29].

The treatment of carcinoma in situ of the cervix has been cold knife conization for many years. The rate of complications of cone excision, particularly regarding fertility, the increase in the number of young nulliparous women diagnosed with CIN and the high cost of hospitalization had been the basis for the birth of destructive methods in local anaesthesia and/or in outpatient regimens, as electrocoagulation diathermy by Chanen *et al.* [30, 31] cryosurgery (reviewed by Di Saia and Creasman) [15], cold coagulation by Duncan [32], CO_2 laser vaporization (reviewed by Puig Tintorè) [33]. All these methods, although possible without or under local anaesthesia by a competent colposcopist when a lesion is colposcopically seen in its entirety, had the disadvantages that were blind since it was not possible to graduate the depth of treatment and also could early and late complications occur. These were the reasons for renouncing destructive methods in favor of excisional methods.

New excisional methods for CIN treatment were introduced by Dorsey and Diggs with carbon dioxide laser conization [34], by Prendiville *et al.* with large loop excision of the transformation zone (LLETZ) [35], and by Ferenczy who in 1994 introduced electroconization with a fine-needle electrode [36].

CIN affects the surface epithelium but can also involve the cervical crypts so that it may be present several mm beneath the surface of the cervix.

In the treatment of CIN three clearances should be obtained: apical, exocervical and lateral. Apical clearance is the disease-free tract of cervical tissue beyond the CIN upper limit; the method to obtain this is preoperative microcolposcopy and an excision beyond the microcolposcopy determined CIN extent. The exocervical clearance is the disease-free tract of cervical tissue lateral to the horizontal CIN extent; the method to obtain this is excision of the entire iodonegative area. Lateral clearance is the disease-free tract of cervical tissue beyond the CIN crypt extent; the method to obtain this is ablation to a depth of 4 mm since the mean crypt involvement does not exceed 3.80 mm [37]. Operatively this is possible using a surgical marker (methylene blue acqueous solution) inserted into the canal with a cytobrush for intraoperative visualization of crypts. The visualization of the stained crypts allows an incision lateral to the crypt [38, 39] . The same depth of excision is used for adenocarcinoma in situ (ACIS) since the depth of crypt involvement varies from 0.5 to 4 mm [40]. Synchronous occurrence of high grade CIN and ACIS has been reported in the literature [41].

The concept of microcarcinoma

In 1947 Mestwerdt [42] introduced the concept of microcarcinoma of the uterine cervix as a specific entity. Since its introduction there have been disagreements concerning the pathologic definition and clinical implications of this disease. The first clear definition was that of the Society of Gynecological Oncology in the USA in 1974: "microinvasive carcinoma is the type of carcinoma in which the neoplastic epithelium invades the stroma to a depth of 3 mm or less measured from the base of the epithelium and in which there is no involvement of the lymphatic or hematic vessels" (quoted by Creasman *et al.*) [43].

The FIGO Cancer Committee announced the new definitions in 1985. Stage Ia was defined as "preclinical carcinoma of the cervix diagnosed only by microscopy" and differentiated it into two varieties: Stage Ia1 and Stage Ia2. Stage Ia1 is "minimal microscopically evident stromal invasion" and Stage Ia 2 is "lesion detected microscopically that can be measured". The upper limit of the measurement should not show a depth of invasion of more than 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates, and a second dimension, the horizontal spread, must not exceed 7 mm. Larger lesions should be staged as Stage Ib.

In notes appended to staging, inter alia, it was stated that the diagnosis of both Stages Ia1 and Ia2 should be based on microscopic examination of removed tissue, preferably a cone, which must include the entire lesion. Vascular space involvement, either venous or lymphatic, should not alter the staging, but should be specifically recorded as it may affect treatment decisions in the future [44].

In 1995 the FIGO Committtee on Gynecologic Oncology, since in the previous classification Stage Ia1 had not been quantified but defined only descriptively, and in an attempt to better quantify early invasive disease of the cervix, specified the cervical cancer staging [45].

Stage Ia was defined as "Invasive cancer identified only microscopically. All gross lesions even with superficial invasion are Stage Ib cancers. Invasion is limited to measured stromal invasion with maximum depth of 5 mm and no wider than 7 mm. The depth of invasion should not be more than 5 mm taken from the base of the epithelium, either surface or glandular, from which it originates. Vascular space involvement, either venous or lymphatic, should not alter the staging".

Stage Ia was sub-classified in: *Stage Ia1:* Measured invasion of stroma no greater than 3 mm in depth and no wider than 7 mm and *Stage Ia2:* Measured invasion of stroma greater than 3 mm and no greater than 5 mm and no wider than 7 mm.

The International Federation for Cervical Pathology and Colposcopy and the Colposcopic classification

In 1972 the First World Congress of Colposcopy and Cervical Pathology was organized at Mar del Plata in Argentina with Maclean as Chair and with the presence of the following authorities: Chanen, Coppleson, Stafl, Hamperl, Mestwerdt, Hermansson, Jakob, Jordan, Wespi, Mc Indoe, Kolstad, Bonilla Musoles and many others. During this Congress the International Federation for Cervical Pathology and Colposcopy (IFCPC) was founded the 6th of November.

One of the numerous objects of the Federation was to contribute to the standardization of terminology and evaluation of diagnostic and therapeutic procedures in the field of cervical pathology.

In 1975, during the II World Congress of Cervical Pathology and Colposcopy of IFCPC held in Graz with Burghardt as Chair, the terminology was standardized and an international nomenclature of colposcopic findings was introduced. In this classification the term "atypical transformation zone" encompassed all suspicious patterns, thus requiring appropriate histological evaluation. The classification was published by Stafl in Obstetrics and Gynecology in 1976 [46].

However, with subsequent correlations, it became obvious that the overall incidence of significant abnormal histologic findings was low and therefore this terminology was questioned as being too imprecise and misleading.

A further specific Nomenclature Committee was constituted by the IFCPC in 1987 at the 6th World Congress held in San Paulo, Brazil. This Committee, under the chairmanship of Stafl was entrusted with the task of developing basic terminology of colposcopic findings which would suitably describe the colposcopic features before and after application of acetic acid and iodine solutions. These were to be descriptive terms only of what might be seen under magnification. Early in their deliberations, there were significant differences in opinion among members of the committee. After three years of deliberation, terminology was finally established and in 1990 during the 7th World Congress of the IFCPC in Rome was approved. The classification was published in 1991 by Stafl and Wilbanks as the writing committee on Obstetrics and Gynecology [47].

The principal advantage of the new international classification was the introduction of a system of grading based on entities of epithelial changes and vascular atypia, similar to that proposed in 1978 by Coppleson, Pixley and Reid [48] and suggested in 1987 by Mossetti, De Palo *et al.* [49]. Another advantage was the elimination of the term "atypical" pertaining to the transformation zone, since a significant disparity when correlated with histology often occurred. Adopting the term abnormal was more appropriate than the term atypical, as it was claimed that the word "abnormal" reflected a more direct opposite to that of normal.

However, there are still criticisms of the new IFCPC colposcopic classification. The first criticism is the use of the same classification for vaginal lesions. It is true that the term colposcopy derives from the Greek word kolpos which means womb, uterus and cavity. Since kolpos means also cavity, the vagina might be considered in the classification. This is however not correct since in the vagina there is no transformation zone. The second criticism is the concept of "within" and "outside" the transformation zone. In changes occurring outside the transformation zone, all cervical lesions are those of HPV infection. Another criticism is the term "erosion" described under the classification as a "major change". The term erosion implies a failure of the superficial layer of the squamous epithelium due to different causes. It is true that within an abnormal transformation zone erosion may be indicative of a high grade lesion, but erosion may also be a benign image. Other criticisms are the significance of ambiguous terms, as "micropapillary or microconvoluted acetowhite epithelium" and "non-acetowhite micropapillary surface". Micropapillary or microconvoluted acetowhite epithelium usually signifies HPV infection, while the second term really does not correspond to a specific colposcopic image.

Therefore a new committee on colposcopic nomenclature was activated at the 10th World Congress of IFCPC in Buenos Aires in 1999 with P. Walker (UK) as Chairman and S. Dexeus (Spain), G. De Palo (Italy), R. Barrasso (France), M. Campion (Australia), F. Girardi (Austria), C. Jakob (Argentina), M. Roy (Canada) as members. At the 11th IFCPC Congress in Barcelona in 2002, this classification introducing some changes in the previous classification was presented. The colposcopy terminology is published in Obstetrics and Gynecology in the first number of 2003 [50].

Cytological classification

The five classes of Papanicolaou have been used for years, but the Papanicolaou classification has demonstrated deficencies because it does not reflect the current understanding of cervical/vaginal neoplasias and has no equivalent in diagnostic histopathologic classification. For these reasons, the Division of Cancer Prevention and Control of the National Cancer Institute of Bethesda convened a workshop of expert consultants to review the existing terminology and to recommend an effective method of reporting. The workshop met at the National Institute of Health in Bethesda from December 12-13, 1988.

The participants at the workshop unanimously affirmed that the Papanicolaou classification was not accep-

table in the modern practice of diagnostic cytopathology; the comunication of cytopathologic findings to the referring physician should be in unambiguous diagnostic terms that have clinical relevance. Therefore a new system should be necessary as a guide-line for cytopathology reports.

The participants at the workshop introduced the concepts of "low-grade squamous intraepithelial lesion" and "high-grade squamous intraepithelial lesion". In the low-grade squamous intraepithelial lesion, CIN I and cellular changes associated with HPV were introduced. The high-grade squamous intraepithelial lesions were comprehensive of CIN II and III. Furthermore, the terms of atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells of undetermined significance (AGUS) were introduced to explain those cases in which the cytological findings were of undetermined significance. These terms should not be used as a diagnosis for otherwise defined inflammatory or pre-neoplastic cellular changes, but should include a recommendation to the physician for further evaluation that may help in determining the significance of atypical cells.

This new cytological classification, the Bethesda System, for reporting cervical/vaginal cytological diagnoses, was published in 1989 in JAMA [51]. The classification was refined in 1991 in a second workshop of the National Cancer Institute of Bethesda [52] in which it was suggested that the diagnosis of atypical cells of undetermined significance should be clarified as "whether a reactive or a premalignant-malignant process is favored" and in 2001 [53] in which atypical squamous cells of undetermined significance have finally been subdivided into two categories: atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells that cannot exclude high-grade SIL (ASC-H).

Condylomatous lesions in the cervix

In 1976, Meisels and Fortin from Laval University of Quebec determined that koylocytosis, a term introduced by Koss and Durfee in 1956 [54], was the characteristic cellular pattern diagnostic for condylomatous lesions [55], and that this morphologic expression was consistently diagnosed as mild dysplasia [56]. In 1978 Della Torre from the National Cancer Institute of Milan reported that intranuclear viral particles morphologically identical with papovavirions were observed in koylocytotic epithelial cells by electromicroscopy [57].

At the start of the 1980s the sexually transmitted agent hypothesized more than 150 years earlier by Rigoni Stern as being responsible for cervical cancer was identified in the human papilloma virus.

Human papillomaviruses are DNA viruses with a diameter of 55 nm, a genome of 8,000 base pairs. They are species-specific, epitheliotropic, resistant to organic solvents and to heating of 58°C and non-cultivable.

To date, more than 80 distinct types of HPV have been described. They are referred to as genotypes because their classification depends on DNA composition. An HPV is considered a new type when its E6, E7, L1 gene sequences (about one-third of the genome) differ by more than 10% from those of any previously known HPV types [58]. They are divided into cutaneous types, cutaneous/epidermodysplasia verruciformis types, cutaneous and/or mucosal types, and mucosal types [58]. More than 30 HPV types may affect genital sites.

In biopsies from cervical carcinoma the team of Zur Hausen identified HPV 16 DNA [59] and HPV 18 DNA [60] whose transforming proteins were identified as E6 and E7 proteins [61]. Afterwards the National Cancer Institute of Bethesda demonstrated that E7 of high-risk HPV binds the tumor suppressor gene RB [62] and E6 binds and degrades the tumor suppressor gene p53 [63].

In 1995 Bosch and the International Biological Study on Cervical Cancer (IBSCC) study group [64] published a report in which more than 1,000 specimens from patients with cervical cancer (squamous, adenocarcinoma, adenosquamous) collected from 32 hospitals in 22 countries (Africa, Central and South America, Southeast Asia, North America and Europe) were studied using PCR assays capable of detecting more than 25 HPV types. HPV DNA was detected in 93% of the carcinomas without significant variations in HPV positivity among the different countries. The most common type in all countries was HPV 16 which was present in 50% of the specimens.

At a later time, a re-examination of these data was performed by the IBSCC and showed that HPV prevalence in cervical invasive carcinoma was 99.7% [65].

Following the data of the IBSCC the International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risk to Humans revised the human papillomaviruses in June 1995. The

IARC program on the evaluation of carcinogenic risk to humans considered the following categories: Category 1 - where there is sufficient evidence for carcinogenicity in humans, the agent being classified as *carcinogenic to humans*; Category 2A - where there is limited evidence of carcinogenicity in humans but adequate evidence in experimental animals, classified as *probably carcinogenic to humans*. Category 2B applies where limited evidence of carcinogenicity in humans exists and there is less than sufficient evidence of carcinogenicity in experimental animals and classified as *possibly carcinogenic to humans*; Category 3 - where there is no classifiable evidence; Category 4 - where there is lack of evidence of carcinogenicity in both humans and experimental animals, classified as *probably not carcinogenic to humans.*

The Working Group of IARC [66], classified HPV 16 and 18 as category 1, HPV 31 and 33 as category 2A, HPV types other than 16, 18, 31, 33 as category 2B, and HPV 6 and 11 as category 4. An update of the conclusions of IARC Monograph 64 was reported by the IBSCC at the 18th International Papillomavirus Conference in Barcelona in July 2000. The IBSCC write "In addition to HPV 16 and 18, HPVs 31, 33, 35, 45, 51, 52, 58, and 59 can now be considered as carcinogenic" [67]. In a further publication, Munoz and the Multicenter Cervical Cancer Study Group of IARC [68] reported that 15 HPV types are classified as high-risk types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) and three as probably high-risk types (HPV 26, 53, 66). Therefore at least 15 types have been adequately evaluated as high-risk in relation to invasive cervical cancer. Furthermore, it is worth noting that there are intra-type variants of HPV 16 (being intratype variants defined as HPVs that vary by 2% or less in specific regions of the genome) [69]. As reported by Yamada *et al.* [70], there are six variants: European (E), Asian (As), African 1 (Af1), African 2 (Af2), North America 1 (NA1), Asian-American (AA). In Europe and North America, E variant predominated (84-93%), in Africa, Af1 and Af2 made up the large majority (92%), in Southeast Asia the As variant was found mainly, in Central and South America the AA variant was important contrary to the other countries. Although differences have been reported for HPV 16 natural variants suggesting that women with HPV 16 non prototype-European variant are at higher risk of developing high-grade CIN than those with E prototype-like variant [71], since almost all researches have utilized the HPV 16 European variant, the clinical significance of the variants are still unclear and longitudinal studies are necessary to estimate the risk of each variant.

Vaccination and the future

The identification of oncogenic HPVs as the causal factor in cervical precancer and cancer implies that development of an effective vaccine against high-risk HPV could prevent the premalignant and malignant disease associated with HPV infection and also stimulate the immune system to produce specific antibodies against the transformation proteins E6 and E7, as first reported by Borysiewicz *et al.* [72].

Preliminary studies in experimental animals and humans have shown promise in the development of a prophylactic vaccine.

The immunogenicity of papillomaviruses involves presentation to the immune system of empty viral capsids composed of L1, the major structural viral protein. Empty (absence of other viral gene products) viral capsids termed "virus-like particles" (VLPs) [73-75] have the ability to generate type-specific neutralizing antibodies, therefore vaccination with L1 VLPs derived from species-specific papillomaviruses neutralizes virus.

In animal models, preventive papillomavirus vaccine using L1 VLPs have been conducted by the use of oral mucosal bovine papillomavirus 4 (BPV4), cutaneous cottontail rabbit papillomavirus (CRPV), and canine oral papillomavirus (COPV) disease. Intramuscular injections in the natural host of BPV4 L1 VLPs, CRPV L1 VLPs, COPV L1 VLPs protected the respective animals against infection [76-79].

In humans two early clinical studies have demonstrated that the HPV-16 L1 VLPs vaccines were well tolerated and generated high levels of antibodies against HPV-16 [80, 81].

The first study is a small, double-blind, randomized, placebo-controlled, dose-escalation, phase I trial conducted at the Johns Hopkin's University Center in Baltimore to evaluate the safety and the immunogenicity of a human papillomavirus type 16 L1 VLPs vaccine in healthy adults. Volunteers received intramuscular injections of placebo or HPV 16 L1 VLPs vaccine given at months 0,1,4. The results showed that this vaccine was well tolerated and was highly immunogenic [80]. The adverse effects such as pain and irritation in the site of injection and a mild hyperthermia were similar in the two arms of treatments.

In a double-blind, multicenter, randomized clinical trial in the USA, recently published by Koutsky *et al.* [81] young (16-23 years of age) women, who were negative for HPV-16 DNA, were randomly assigned to receive by intramuscular injections three doses of HPV-16 L1 VLPs vaccine or placebo on day 0, month 2 and month 6. With a median follow-up of 17.4 months after completing the vaccination regimen, 41 cases of new HPV-16 infections, including nine cases of HPV-16 related CIN occurred among placebo recipients versus 0 in the group with HPV-16 vaccine [81].

The central issue is the number of viral types that should be included in the preventive vaccination. The cumulative prevalence of HPV types in the recent study of Munoz *et al.* [68] showed that HPV types 16, 18, 45, 31, 33, 52, 58 and 35 were in descending order of frequency the eight most common types in cervical cancer. Therefore a polyvalent HPV vaccine should be established.

A prophylactic polyvalent vaccine against oncogenic HPVs in young girls prior to the onset of sexual activity is the future in which cervical cancer will be not only a predictable, preventable and curable disease, but it will be an avoidable disease, so that as the Latin poet Horatius Flaccus said "quem non habére sperabas diem, grato animo àccipiet" ("you will accept with gratitude the day that you did not wish to have").

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