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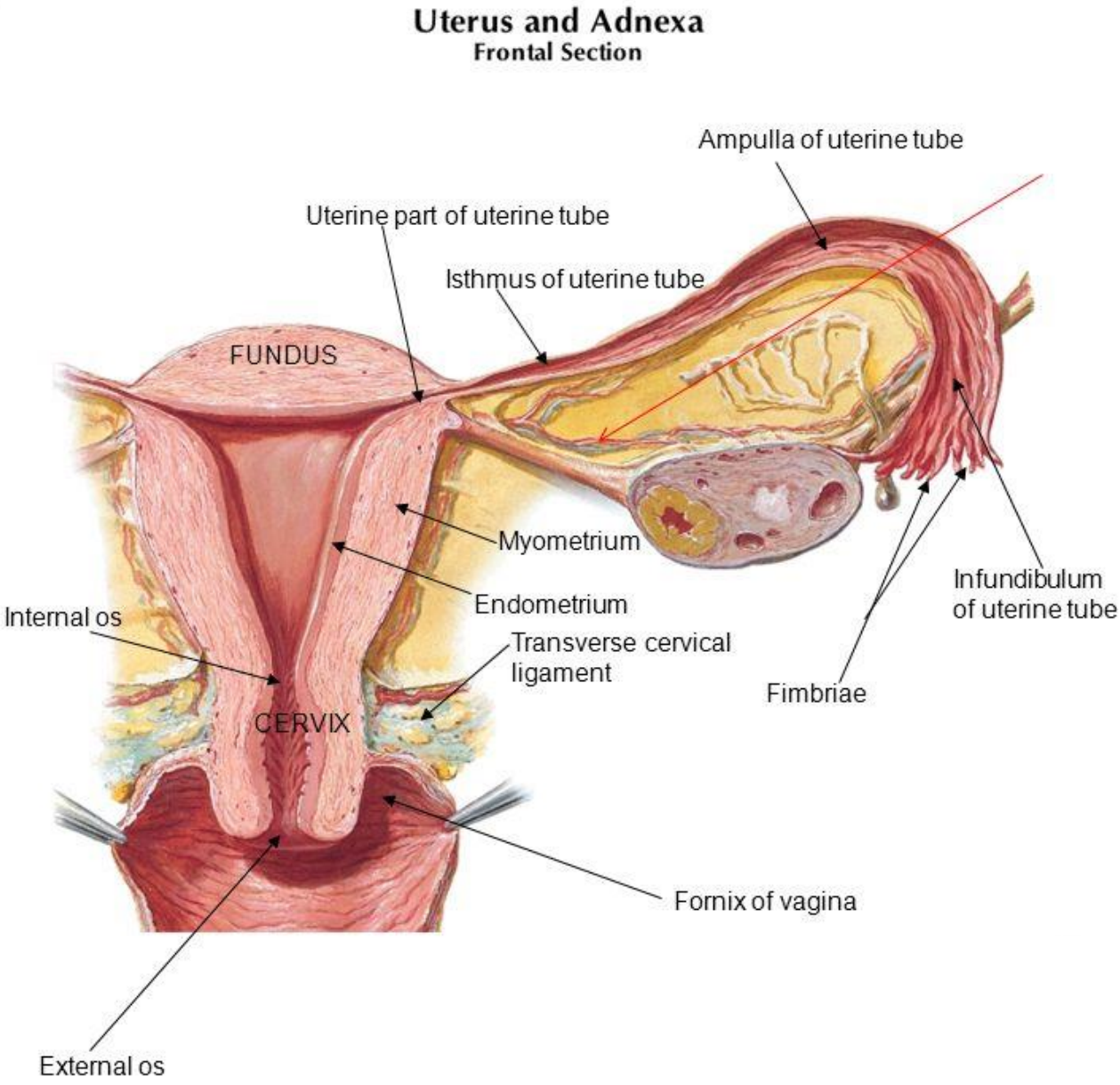
CHAPTER I

1.2 PRE-INVASIVE AND INVASIVE CERVICAL CANCER

1.1.1 Anatomy of the uterine cervix

The cervix is a narrow, cylindrical segment of the uterus; it enters the vagina through the anterior vaginal wall and lies, in most cases, at a right angle to it. In the average patient, the cervix measures 2 to 4 cm in length and is contiguous with the inferior aspect of the uterine corpus. The point of juncture of the uterus and the cervix is known as the isthmus; this area is marked by slight constriction of the lumen. Anteriorly, the cervix is separated from the bladder by fatty tissue and is connected laterally to the broad ligament and parametrium (through which it obtains its blood supply). The lower intravaginal portion of the cervix, a free segment that projects into the vault of the vagina, is covered with mucous membrane. The cervix opens into the vaginal cavity through the external os. The cervical canal extends from the anatomic external os to the internal os, where it joins the uterine cavity. The histologic internal os is where there is a transition from endocervical to endometrial glands. The intravaginal portion of the cervix (portio vaginalis, exocervix) is covered with stratified squamous epithelium that is essentially identical to the epithelium of the vagina. The endocervical mucosa is arranged in branching folds (plicae palmatae) and is lined by cylindrical epithelium. The stroma of the cervix consists of connective tissue with stratified muscle fibers and elastic tissue. The elastic tissue is found primarily around the walls of the larger blood vessels. The stratified squamous epithelium of the portio vaginalis is composed of several layers that are conventionally described as basal, parabasal, intermediate, and superficial. The basal layer consists of a single row of cells and rests on a thin basement membrane. This is the layer in which active mitosis occurs. The parabasal and intermediate layers together constitute the prickle-cell layer, which is analogous to the same layer in the epidermis. The superficial layer varies in thickness, depending on the degree of estrogen stimulation.


Uterus and Adnexa
Frontal Section
Ampulla of uterine tube
Uterine part of uterine tube
Isthmus of uterine tube
FUNDUS
Myometrium
Endometrium
Internal os
Transverse cervical ligament
CERVIX
Infundibulum of uterine tube
Fimbriae
Fornix of vagina
External os

1.1.2 Epidemiology of cervical cancer

In the United States the mortality from cervical cancer in 1945 was 15 of 100,000 females. This had declined to approximately 4.6 of 100,000 by 1986 and 3.4 of 100,000 by 1991. It is unclear whether the mortality from cervical cancer is falling as a result of cervical cytologic screening and intervention at the in situ stage or whether cervical screening has caused an increase in the proportion of early-stage cancer at diagnosis and registration[1]. Arbyn et al. in 2018 extracted e estimated number of cases of and deaths from cancer of the cervix uteri (International Classification of Diseases tenth edition [ICD-10] code C53) in 185 countries in 2018 from the Global Cancer Observatory (GLOBOCAN) 2018 database, as published by the IARC[2,3]. Authors calculated the directly age-standardised incidence rate (ASIR) and agestandardised mortality rate (ASMR) using the world standard population. In 2018, approximately 570 000 women developed cervical cancer and 311 000 women died from it, corresponding to an all-ages ASIR of 13·1 per 100 000 women-years and ASMR of 6·9 per 100 000. Worldwide, cervical cancer was the fourth most common cancer among women, after breast cancer (2·09 million cases), colorectal cancer (0·79 million), and lung cancer (0·73 million); and it was also the fourth leading cause of cancer death among women, after breast (627 000 deaths), lung (576 000) and colorectal (387 000) cancers. Approximately 84% of all cervical cancers and 88% of all deaths caused by cervical cancer occurred in lower-resource countries **Table 1 and Table 2**.

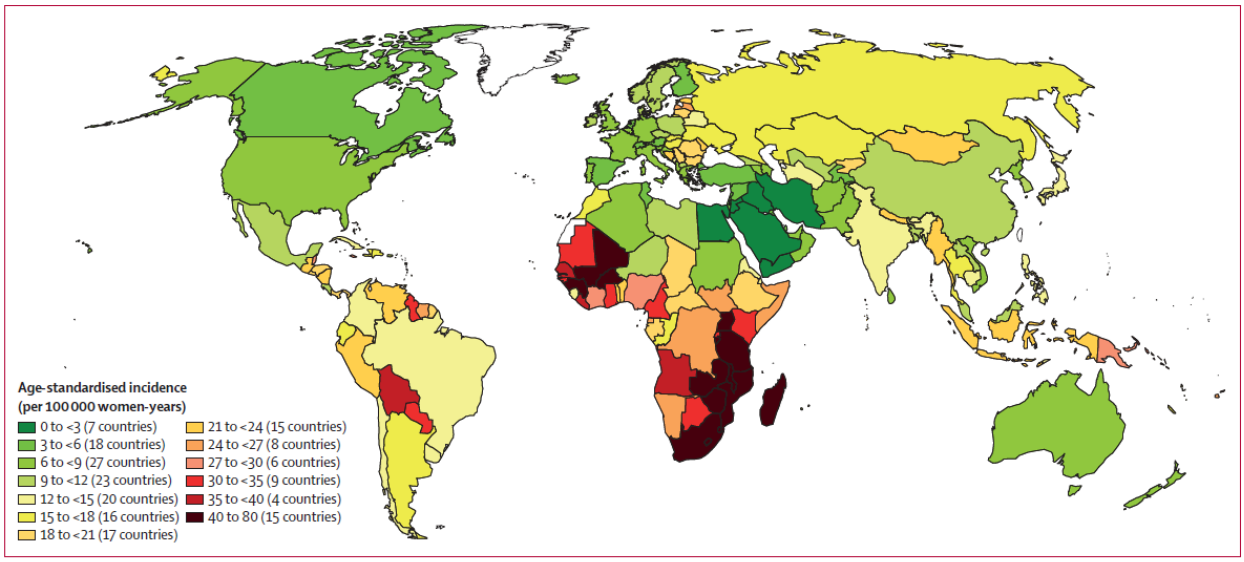


Figure 1: Geographical distribution of world age-standardised incidence of cervical cancer by country, estimated for 2018

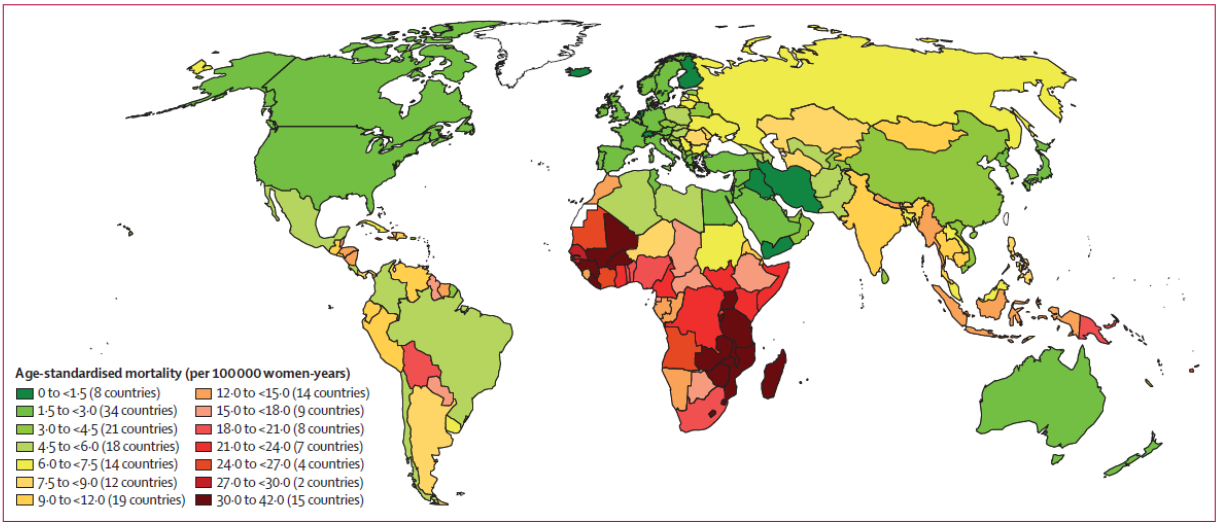


Figure 2: Geographical distribution of world age-standardised mortality rate of cervical cancer by country, estimated for 2018

According to this analysis, the lowest incidence burden was observed in western Asia and the lowest mortality burden was observed in Australia–New Zealand. Rather modest incidences (ASIR <10 per 100 000) were also noted in Australia–New Zealand, northern America, western Europe, northern Africa, southern Europe, and northern Europe. The highest burden was observed in southern Africa and eastern Africa. A very high burden of the disease (ASIR ≥15 per 100 000) was also observed in western Africa, Melanesia, middle Africa, Micronesia, southeastern Asia, eastern Europe, the Caribbean, and South America. The highest incidences (ASIR >40 per 100 000) were all found in countries from eastern, southern, or western Africa (eSwatini , Malawi, Zambia, Zimbabwe, Tanzania, Burundi, Uganda, Lesotho, Madagascar, Comoros, Guinea, Burkina Faso, Mali, South Africa, and Mozambique; figure 3). China was the country with the highest number of cases (106 000), whereas India was the country with the highest estimated number of cervical cancer deaths (60 000). China and India together contributed 35% to the global burden of cervical cancer cases and deaths. With almost 0.6 million cases and 0·3 million deaths per year, cervical cancer continues to constitute a major public health problem, ranking as the fourth most common cause of cancer incidence and mortality in women worldwide.

1.1.3 Histological types of cervical cancer

Cervical cancer is divided into two main types: squamous cell carcinoma and adenocarcinoma. Each is distinguished by the appearance of cells under a microscope.

Squamous cell carcinomas begin in the thin, flat cells that line the bottom of the cervix. This type accounts for about 90 percent of cervical cancers, according to the ACS.

Adenocarcinomas of the cervix develop in the glandular cells that line the upper portion of the cervix. Cervical adenocarcinomas make up most of the remaining cervical cancer cases.

In most large series, approximately 85% to 90% of malignant lesions of the cervix are squamous cell, but other lesions are possible.

TABLE 3-4 Histologic Classification of Cervical Cancer

Epithelial Tumors		
Nonglandular	**Glandular**	**Other, Including Mixed**
Squamous cell carcinoma	Adenocarcinoma, usual endocervical type	Adenosquamous
Verrucous carcinoma	Mucinous adenocarcinoma	Glassy cell carcinoma
Warty (condylomatous) carcinoma	Endometrioid adenocarcinoma	Mucoepidermoid carcinoma
Papillary squamotransitional carcinoma	Well-differentiated villoglandular adenocarcinoma	Adenoid cystic carcinoma
Lymphoepithelial-like carcinoma	Adenoma malignum (minimal deviation)	Adenoid basal carcinoma
Sarcomatoid carcinoma	Intestinal-like adenocarcinoma	Small cell carcinoma
	Signet ring cell adenocarcinoma	Classical carcinoid tumor
	Colloid adenocarcinoma	Gestational choriocarcinoma
	Clear cell adenocarcinoma	
	Serous papillary adenocarcinoma	
	Mesonephric adenocarcinoma	
Nonepithelial Tumors		
Mesenchymal Tumors	**Germ Cell Tumors**	**Miscellaneous**
Carcinosarcoma	Mature teratoma	Melanoma
Leiomyosarcoma	Immature teratoma	Lymphoma
Epithelioid leiomyosarcoma	Yolk sac tumor	Primitive neuroectodermal tumor
Extrauterine endometrial stromal sarcoma	Nongestational choriocarcinoma	
Adenosarcoma		
Embryonal rhabdomyosarcoma		
Granulocytic sarcoma (chloroma)		

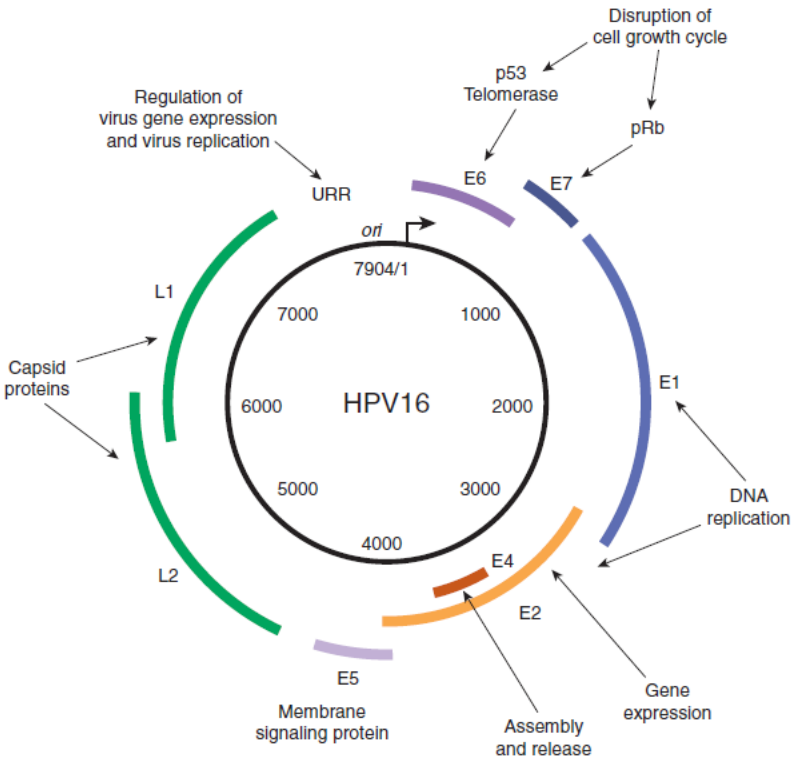
From Tewari KS, Monk BJ: Tumors of the cervix. In: Raghavan et al, eds. Textbook of Uncommon Cancer. Ed 3. Hoboken, NJ, 2006, John Wiley & Sons, Ltd.

1.1.4 The role of Human Papillomavirus infection in the etiology of cervical cancer

Human papillomaviruses (HPVs) are non-enveloped viruses consisting of an icosahedral capsid of about 60 nm in diameter, with a double stranded circular DNA of approximately 8000 base pairs[4]. HPVs contain three genomic regions, including approximately ten open reading frames (ORFs). Polycistronic mRNAs generate many of the viral proteins. The viral genome can be subdivided into three regions, including the early region (E), with up to seven ORFs encoding viral regulatory proteins; the late region (L), that encodes the two viral capsid proteins; and the long control region (LCR), or upstream regulatory region (URR), composed by the origin of replication and transcription control sequences.

The main drivers of carcinogenesis, which discriminates High-Risk (HR) and Low-Risk (LR) HPVs, are the early proteins E6, E7, and E5. Both HR and LR E6 and E7 proteins can interact respectively with p53 and retinoblastoma protein (pRb), but only HR HPVs are able to induce their degradation and inactivation.

Virtually all cervical cancer cases are triggered by persistent infection of the uterine cervix by HPV HR genotypes, particularly HPV16 and HPV18[5]. However, it is known that the virus alone is not sufficient to cause this malignant disease. Dysregulation of both viral and host gene expression due to viral DNA integration into the cell's genome, as well as epigenetic modifications are crucial events in the carcinogenic process[6]. In addition, high-risk HPV infection can lead to aberrant expression of oncogenic and tumor suppressor micro RNAs (miRNAs), most of which have either c-Myc, p53 or E2F transcription factors as downstream targets, and whose expression can be modulated by the E6 and E7 viral oncoproteins[7-8]. Although HPV is a necessary cause of cervical cancer, it is not a sufficient cause. Thus, other cofactors are necessary for progression from cervical HPV infection to cancer. Long-term use of hormonal contraceptives, high parity, tobacco smoking, and co-infection with HIV have been identified as established cofactors; co-infection with Chlamydia trachomatis (CT) and herpes simplex virus type-2 (HSV-2), immunosuppression, and certain dietary deficiencies are other probable cofactors. Genetic and immunological host factors and viral factors other than type, such as variants of type, viral load and viral integration, are likely to be important but have not been clearly identified.

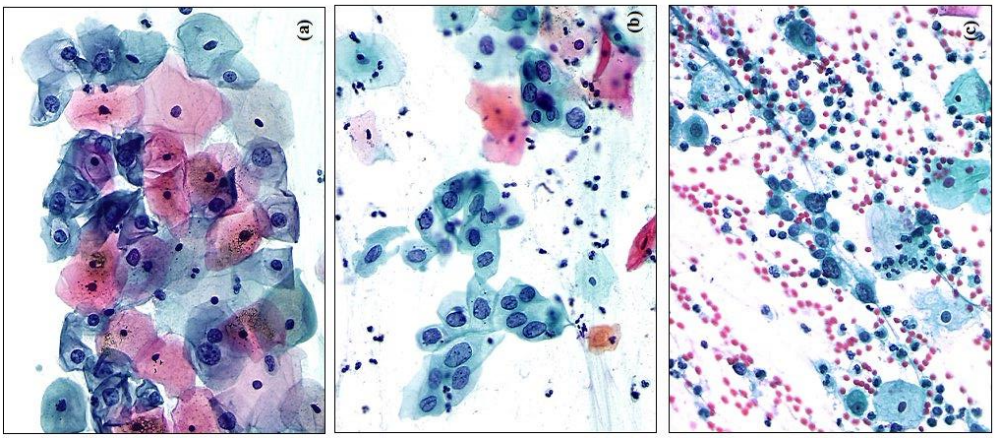


Human papillomavirus genome

Invasive cervical cancers are usually preceded by a long phase of preinvasive disease. This is characterized microscopically as a spectrum of events progressing from cellular atypia to various grades of dysplasia or cervical intraepithelial neoplasia (CIN) before progression to invasive carcinoma. A system of classification with separate classes for dysplasia and carcinoma in situ (CIS) was increasingly perceived as an arbitrary configuration, based upon the findings from cases of follow-up studies involving women with such lesions. It was observed that some cases of dysplasia regressed, some persisted, and others progressed to CIS. A direct correlation with progression and histological grade was observed. These observations led to the concept of a single, continuous disease process by which normal epithelium evolves into epithelial precursor lesions and on to invasive cancer. On the basis of the above observations, the term cervical intraepithelial neoplasia (CIN) was introduced in 1968 to denote the whole range of cellular atypia confined to the epithelium. CIN was divided into grades 1, 2 and 3 (Richart 1968). CIN 1 corresponded to mild dysplasia, CIN 2 to moderate dysplasia, and CIN 3 corresponded to both severe dysplasia and CIS. In the 1980s, the pathological changes such as koilocytic or condylomatous atypia associated with human papillomavirus (HPV) infection were increasingly recognized. Koilocytes are atypical cells with a perinuclear cavitation or halo in the cytoplasm indicating the cytopathic changes due to HPV infection. This led to the development of a simplified two-grade histological system. Thus, in 1990, a histopathological terminology based on two grades of disease was proposed: low-grade CIN comprising the abnormalities consistent with koilocytic atypia and CIN 1 lesions and high-grade CIN comprising CIN 2 and 3. The high-grade lesions were considered to be true precursors of invasive cancer (Richart 1990).

1.1.5 Pre-invasive cervical lesions

CIN may be identified by microscopic examination of cervical cells in a cytology smear stained by the Papanicolaou technique. In cytological preparations, individual cell changes are assessed for the diagnosis of CIN and its grading. Nuclear enlargement with variation in size and shape is a regular feature of all dysplastic cells. Increased intensity of staining (hyperchromasia) is another prominent feature. Irregular chromatin distribution with clumping is always present in dysplastic cells. Mitotic figures and visible nucleoli are uncommon in cytological smears. Abnormal nuclei in superficial or intermediate cells indicate a low-grade CIN, whereas abnormality in nuclei of parabasal and basal cells indicates high-grade CIN. The amount of cytoplasm in relation to the size of the nucleus (nuclear-cytoplasmic ratio) is one of the most important bases for assessing the grade of CIN. Increased ratios are associated with more severe degrees of CIN.



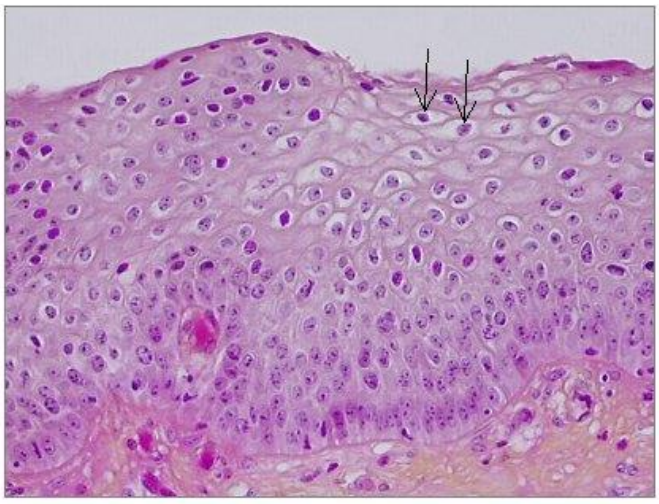
Cytological appearance of (a) CIN 1, (b) CIN 2, (c) CIN 3 (x20)

Final diagnosis of CIN is established by the histopathological examination of a cervical punch biopsy or excision specimen. A judgement of whether or not a cervical tissue specimen reveals CIN, and to what degree, is dependent on the histological features concerned with differentiation, maturation and stratification of cells and nuclear abnormalities.

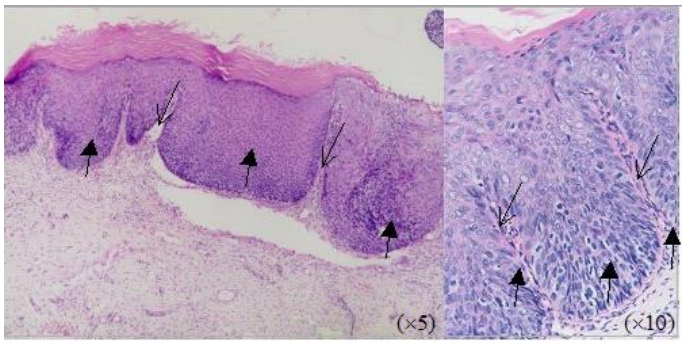
In CIN 1 there is good maturation with minimal nuclear abnormalities and few mitotic figures. Undifferentiated cells are confined to the deeper layers (lower third) of the epithelium. Mitotic figures are present, but not very numerous. Cytopathic changes due to HPV infection may be observed in the full thickness of the epithelium.

CIN 2 is characterized by dysplastic cellular changes mostly restricted to the lower half or the lower two-thirds of the epithelium, with more marked nuclear abnormalities than in CIN 1. Mitotic figures may be seen throughout the lower half of the epithelium.

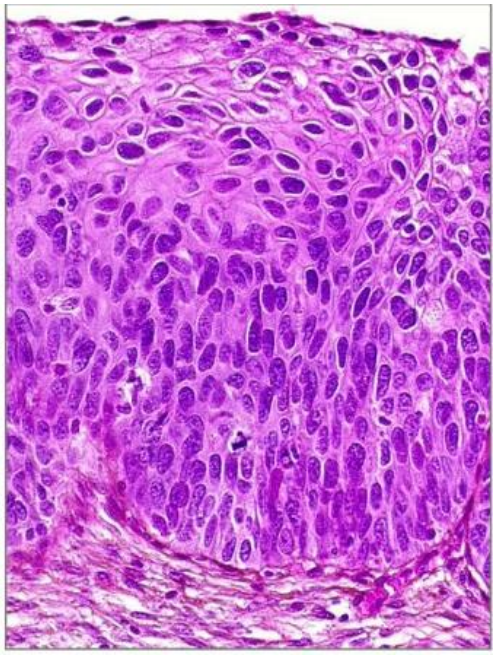
In CIN 3, differentiation and stratification may be totally absent or present only in the superficial quarter of the epithelium with numerous mitotic figures. Nuclear abnormalities extend throughout the thickness of the epithelium. Many mitotic figures have abnormal forms.



Histology of CIN 1: Note that the dysplastic cells are confined to the lower third of the epithelium. Koilocytes indicated by arrows are observed mostly in the upper layers of the epithelium (x20)



Histology of CIN 2: Atypical cells are found mostly in the lower two-thirds of the epithelium. Note the rete pegs indicated by the heavy arrows. Note the stretched out capillaries in the stromal papillae indicated by the narrow arrows



Histology of CIN 3: Dysplastic cells are distributed in the upper third of the epithelium in addition to the lower two-thirds. Note the loss of polarity of cells (x40)

1.1.6 Invasive cervical cancer

The widely used staging system for cervical cancer was developed by the International Federation of Gynecology and Obstetrics (FIGO). Until recently, cervical cancer FIGO 2009 staging was based on clinical evaluation, including physical examination and limited imaging modalities, in order to accommodate practitioners in low-resource countries who might not have access to more extensive pathologic and imaging modalities. Such an approach was deemed increasingly more inadequate given the progressive changes in novel imaging modalities and surgical approaches that became part of the routine management of patients with cervical cancer[9]. This disparity left a gap between an outdated clinical staging and a broad range of information available to the oncologist. To that end, a new FIGO staging classification was proposed in 2018 which implemented, in addition to physical examination, information gathered from imaging modalities and surgical histopathological results[10]. The latest modification of the staging classification allows incorporation of pelvic ultrasound, magnetic resonance imaging (MRI), computed tomography (CT), and/or positron emission tomography/CT (PET/CT) in order to appropriately assign a stage to the patient. In addition, histopathological findings obtained from the surgical specimen—particularly lymph node status—may be used in order to upstage the patient. The FIGO staging is assessed using methods including inspection and palpation by vaginal and rectal examination, colposcopy, cystoscopy, endocervical curettage, hysteroscopy, intravenous urogram, and chest and skeletal X-rays. Lymphangiography, ultrasonography, computerized tomography (CT) and magnetic resonance imaging (MRI) and laparoscopy may provide additional information, but this information should not be used to assess the FIGO clinical stages, even though these investigations may provide valuable information for planning treatment. In many low-resource settings, however, speculum examination, per vaginal and per rectal examination are the only feasible approaches to staging. Cystoscopy and radiological assessment with chest and skeletal X-rays and intravenous urograms may additionally be carried out if possible. This is primarily a clinical staging system based on tumour size and extension of the disease in the pelvis. The extent of growth of cancer is assessed clinically, as well as by various investigations to categorize the disease stages I through IV. Stage I represents growth localized to the cervix, while stage IV represents the growth phase in which the cancer has spread to distant organs by metastases.

Table 3.1: FIGO staging (See Figure 3.10)

Stage I

Stage I is carcinoma strictly confined to the cervix; extension to the uterine corpus should be disregarded. 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.

Stage IA: Invasive cancer identified only microscopically. Invasion is limited to measured stromal invasion with a maximum depth of 5 mm and no wider than 7 mm.

Stage IA1: Measured invasion of the stroma no greater than 3 mm in depth and no wider than 7 mm diameter.

Stage IA2: Measured invasion of stroma greater than 3 mm but no greater than 5 mm in depth and no wider than 7 mm in diameter.

Stage IB: Clinical lesions confined to the cervix or preclinical lesions greater than Stage IA. All gross lesions even with superficial invasion are Stage IB cancers.

Stage IB1: Clinical lesions no greater than 4 cm in size.

Stage IB2: Clinical lesions greater than 4 cm in size.

Stage II

Stage II is carcinoma that extends beyond the cervix, but does not extend to the pelvic wall. The carcinoma involves the vagina, but not as far as the lower third.

Stage IIA: No obvious parametrial involvement. Involvement of up to the upper two-thirds of the vagina.

Stage IIB: Obvious parametrial involvement, but not to the pelvic sidewall.

Stage III

Stage III is carcinoma that has extended to the pelvic sidewall. On rectal examination, there is no cancer-free space between the tumour and the pelvic sidewall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or a non-functioning kidney are Stage III cancers.

Stage IIIA: No extension to the pelvic sidewall but involvement of the lower third of the vagina.

Stage IIIB: Extension to the pelvic sidewall or hydronephrosis or non-functioning kidney.

Stage IV

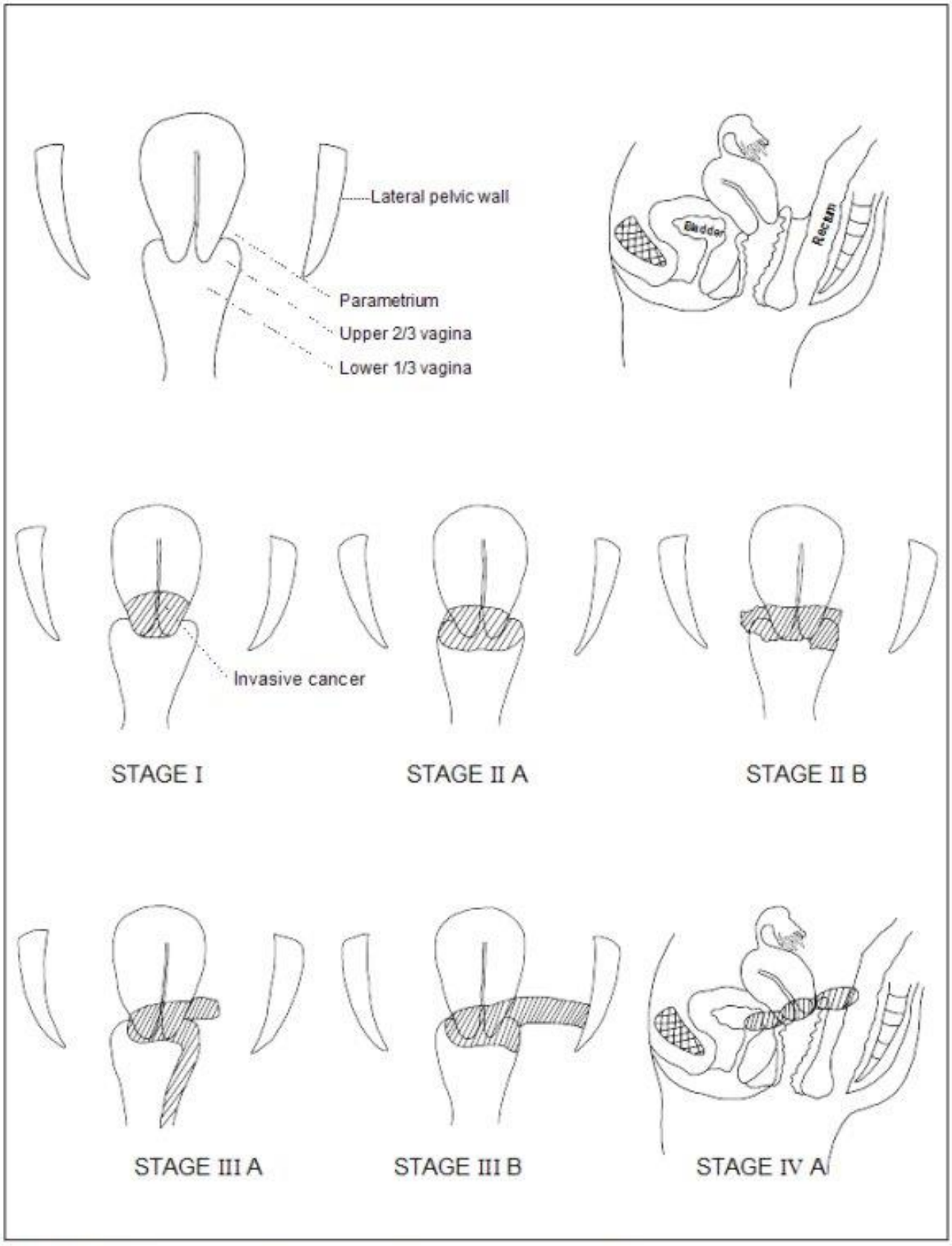


FIGURE 3.10: A schematic diagram of clinical stages of invasive cancer of the cervix.

1.1.7 Cervical cancer screening and treatment

Liquid-based and conventional methods of cervical cytology specimen collection are acceptable for screening. Exfoliated cells are collected from the transformation zone of the cervix and transferred to a vial of liquid preservative that is processed in the laboratory (liquid-based technique) or transferred directly to a slide and fixed (conventional technique)[11]. The liquid-based method of cervical cytology specimen collection has the advantage of allowing a single specimen to be used to perform cytology, HPV testing, and testing for gonorrhea and chlamydial infection. Despite several theoretic advantages of the liquid-based technique, including easier interpretation, filtering of blood and debris, and fewer unsatisfactory results, a meta-analysis of eight studies and a randomized trial did not show an appreciable difference in sensitivity or specificity for the detection of CIN compared with the conventional cervical cytology screening technique[12-13]. The Bethesda System of cervical cytologic test result reporting generally is accepted[14-15].

Interpretation/Results
- Negative for intraepithelial lesion or malignancy
  - Organisms may be identified
  - Other non-neoplastic findings may be noted
    - Inflammation
    - Radiation changes
    - Atrophy
  - Glandular cells status post hysterectomy
  - Atrophy
- Epithelial cell abnormalities
  - Squamous cells
    - Atypical squamous cells (ASC)
      - Of undetermined significance (ASCUS)
      - Cannot exclude HSIL (ASC-H)
    - Low-grade squamous intraepithelial lesions (LSIL)
      - HPV, CIN I
    - High-grade squamous intraepithelial lesions (HSIL)
      - CIN II, CIN III
    - Squamous cell carcinoma
  - Glandular cell
    - Atypical glandular cells (AGC)—specify origin
    - Atypical glandular cells favor neoplastic—specify origin
    - Endocervical adenocarcinoma in situ (AIS)
    - Adenocarcinoma

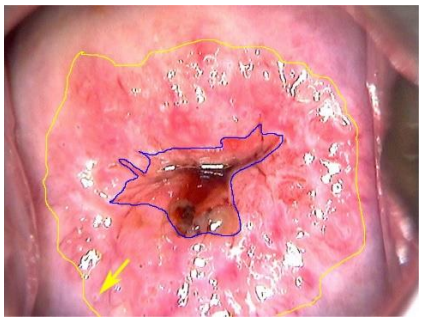
Furthermore, several tests have been approved by the FDA for the detection of cervical HPV. They assess exfoliated cervical cells for the presence of subsets of the potentially cancer-causing HR HPV genotypes (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68)[16]. Testing should be performed only to detect the presence of high-risk HPV. There is no role for testing for low-risk genotypes, and tests for low-risk HPV should not be performed. Cervical cancer screening should begin at age 21 years. With the exception of women who are infected with HIV, women younger than 21 years should not be screened regardless of the age of sexual initiation or the presence of other behavior-

related risk factors[17]. The recommendation to start screening at age 21 years regardless of the age of onset of sexual intercourse is based on the very low incidence of cancer and the lack of data that screening is effective in this age group[18-19].

Women aged 21–29 years should be tested with cervical cytology alone, and screening should be performed every 3 years. Co-testing should not be performed in women younger than 30 years. For women aged 30–65 years, co-testing with cytology and HPV testing every 5 years is preferred; screening with cytology alone every 3 years is acceptable. These screening recommendations are not meant for women with cervical cancer or those who have HIV infection, are immunocompromised, or were exposed to diethylstilbestrol in utero.

Abnormal cervical citology with or without HPV infection represent an indication for colposcopy examination and possible cervical biopsy. Colposcopy was introduced by Hinselman in 1925 (in Hamburg, Germany) as a result of his efforts to devise a practical method of more minute and comprehensive examination of the cervix. Hinselman and others during his era believed that cervical cancer began as miniature nodules on the surface epithelium and that these lesions could be detected with increased magnification and illumination. The meticulous examination of thousands of cases enabled him to clearly define the multiple physiologic and benign changes in the cervix and to correlate atypical changes with preinvasive and early invasive cancer.

The colposcope consists, in general, of a stereoscopic, binocular microscope with low magnification. It is provided with a center illuminating device and mounted on an adjustable stand with a transformer in the base. Several levels of magnification are available, the most useful being between 8× and 18×. A green filter is placed between the light source and the tissue to accentuate the vascular patterns and color tone differences between normal and abnormal patterns. Examination of the epithelium of the female genital tract by colposcopy usually takes no more than a few minutes. Colposcopy is based on study of the transformation zone. The transformation zone is that area of the cervix and vagina that was initially covered by columnar epithelium and, through a process referred to as metaplasia, has undergone replacement by squamous epithelium. The wide range and variation in the colposcopic features of this tissue make up the science of colposcopy.



The cervical trasformation zone

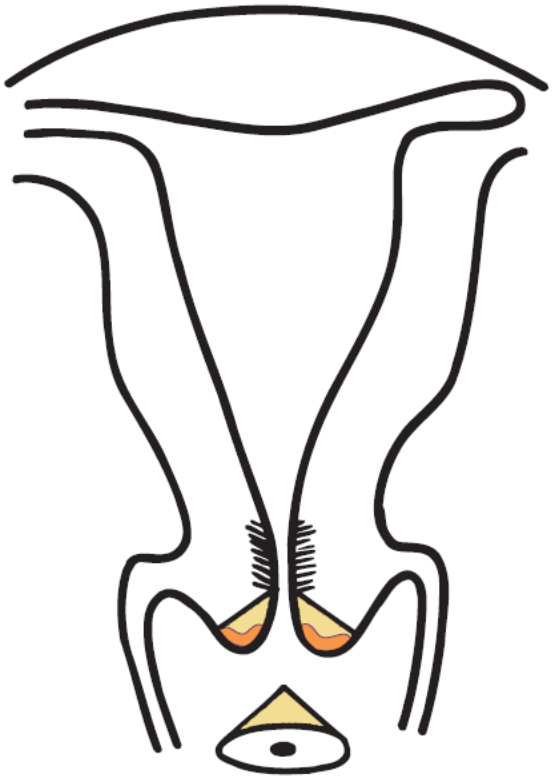
The colposcope

When colposcopy is performed, a standard procedure is followed. First, the cervix is sampled for cytologic screening, and then it is cleansed with a 3% acetic acid solution to remove the excess mucus and cellular debris. The acetic acid also accentuates the difference between normal and abnormal colposcopic patterns. The colposcope is focused on the cervix and the transformation zone, including the squamocolumnar junction, and the area is inspected in a clockwise fashion. In most cases, the entire lesion can be outlined, and the most atypical area can be selected for biopsy. If the lesion extends up the canal beyond the vision of the colposcopist, the patient will require a diagnostic conization to define the lesion. The colposcopy can only suggest an abnormality; final diagnosis must rest on a tissue examination by a pathologist. Selected spot biopsies in the areas showing atypical colposcopic patterns, under direct colposcopic guidance and in combination with cytologic testing, give the highest possible accuracy in the diagnosis and evaluation of the cervix. Probably the greatest value of colposcopy is that in most cases a skilled colposcopist can establish and differentiate invasive cancer from CIN by direct biopsy and thus avoid the necessity of surgical conization of the cervix.

Women with CIN I can be followed without definitive treatment. This is particularly true if the preceding Pap smear result shows ASCUS, ASC-H, or LSIL. These women can be followed with either HPV-DNA testing every 12 months or repeat cytology at 6- to 12-month intervals. If abnormal results remain, further follow-up with repeat colposcopy and treatment if abnormality persists or further observation is acceptable. In women with CIN II or III therapy is indicated. For those with CIN I observation is an appropriate option. Many treatment options are available to the patient today. The most used treatment for pre-invasive cervical lesions is the Loop Electrosurgical Excision Procedure (LEEP). After colposcopy and if the entire transformation zone is identified, it is excised with a low-voltage diathermy loop under local anesthesia. Usually less than 10 mL of local anesthesia, with epinephrine or vasopressin added to help decrease blood loss, is injected into the cervix at 12, 3, 6, and 9 o'clock. After 3 to 5 minutes, excision can be performed with a loop size that will excise the complete lesion. An electrosurgical generator is used with wattage set at 25 to 50, depending on loop size (the larger the loop, the higher will be the wattage) and blended cut or coagulated. The cutting loop consists of an insulated shaft with a wire loop attached. The sterilized steel wire is 0.2 mm in diameter and comes in various sizes.

This technique has several advantages. The procedure can be done on an outpatient basis. Tissue is available for study. Diagnosis and therapy are done at one time and during the same visit. In essentially all large studies reported to date, several early invasive lesions were identified that had not been recognized on colposcopy examination. This technique tends to negate this inherent problem of destructive techniques.

Conization of the cervix or cold knife cone (CKC) is a surgical procedure used to treat or diagnose cervical dysplasia. It is the excision of a cone-shaped portion of the cervix to remove a cervical lesion and the entire transformation zone. A surgeon should perform a diagnostic excisional procedure if there is a lesion that is suspicious for invasive cancer or an adenocarcinoma in situ of the cervix. If there is a histological discordance with the cytological screening test and histological results are less severe, then a diagnostic cone is recommended. Unsatisfactory colposcopic evaluation with evidence of dysplasia present or unexplained high grade or an atypical glandular cell cytology needs an excisional cone or if the entire lesion is not completely visualized on colposcopy. These procedures are also adequate to treat severe dysplasia (CIN 2/3, CIS) and stage 1A1 squamous cell cervical cancer if the patient wants to maintain her fertility.



In cases of invasive cervical cancer, treatment options include surgery, radiotherapy and chemotherapy, and these may be used in combination. A radical hysterectomy is the standard treatment for early-stage cervical cancer. That includes stage I cervical cancer, and more specifically, stage IA2 and IB1[20]. Minimally invasive radical trachelectomy has emerged as an alternative to open radical hysterectomy for patients with early-stage cervical cancer desiring future fertility[21]. Radical hysterectomy and tailored adjuvant radiation therapy in stage IB2 cervical cancer is feasible[22].

1.2 HUMAN IMMUNODEFICIENCY VIRUS INFECTION

1.2.1 Etiology and epidemiology

Human immunodeficiency virus (HIV) infection probably spread from non-human primates to humans sporadically throughout the 1900s[23-24]. However, only in the 1980s did the virus come to the world's attention, when homosexual men in urban centres began presenting with advanced and unexplained immunodeficiency[25].

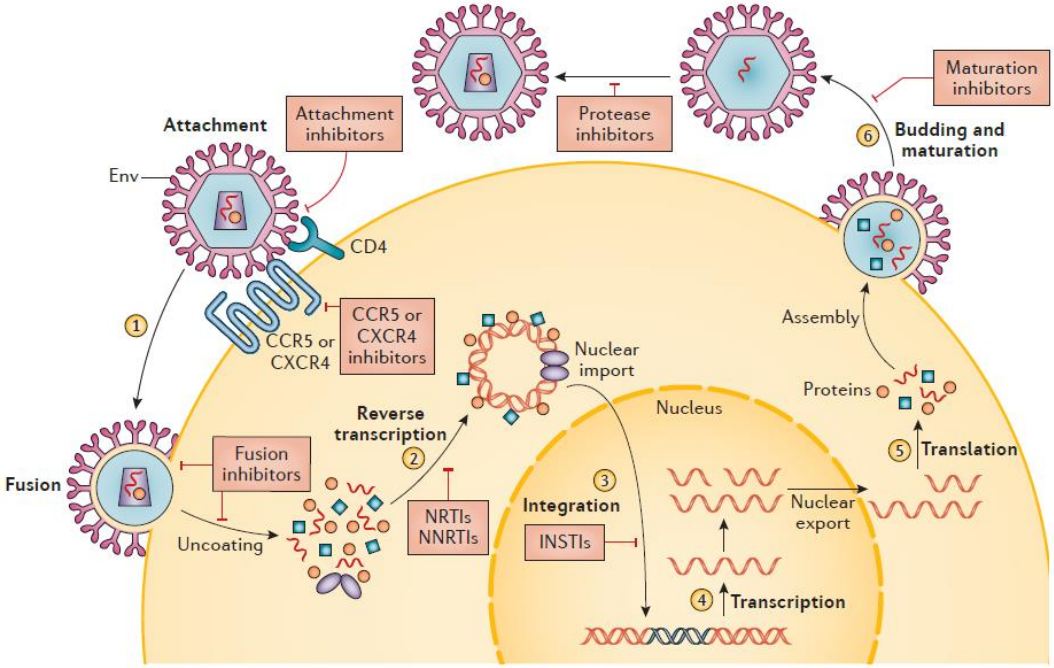
HIV includes a diverse collection of viruses, including HIV type 1 (HIV-1) and HIV-2. HIV-1 is more prevalent and more pathogenic than HIV-2 and is responsible for the vast majority of the global pandemic. Sequence comparisons suggest that both HIV-1 and HIV-2 are the result of cross-species transmissions of simian immunodeficiency virus (SIV) from chimpanzees (SIVcpz) and sooty mangabeys (SIVsmm), respectively.

HIV is a retrovirus and is, therefore, able to integrate its DNA into the host genome; this fact makes the virus exceedingly difficult to eradicate with current therapies[26]. The virus has a small number of proteins and is remarkably efficient in its design. The HIV viral membrane contains 14 'spikes', gp160 complexes consisting of three gp120 proteins each linked to a gp41 protein in a tripod shape. These 'spikes', anchored into the viral membrane by gp41, allow the virus to attach itself onto host cells. The gp160 complex binds with the CD4 receptor on the host cell membrane, then the gp120 associates with a chemokine receptor and dissociates completely from the gp41 allowing the viral membrane to fuse with that of the host.

After gaining entry into a cell, single-strand RNA is reverse transcribed into HIV DNA, which is then integrated into the host DNA. Taking advantage of host enzymes, HIV is transcribed, proteins are produced and cleaved, and mature virions are released. The primary receptor for HIV-1 is CD4, which is expressed on the surface of T lymphocytes, monocytes, macrophages and dendritic cells. HIV also requires a co-receptor to gain entry into the host cell, typically the chemokine receptors CCR5 and CXCR4. Different HIV-1 variants typically use one or the other chemokine receptor, but some can use either; viruses that use these co-receptors for entry are called R5, X4 or R5X4 viruses, respectively. CCR5 and CXCR4 are differentially

expressed on some T cell subsets, with CCR5 expressed at high levels in memory T lymphocytes but not on naive T lymphocytes, whereas CXCR4 is expressed on both. CCR5 is also expressed on macrophages and dendritic cells. The preferred targets for infection are activated T lymphocytes, which for reasons that remain to be defined are more permissive

to infection than resting cells. Although dendritic cells are difficult to infect with HIV-1, they are able to 'capture' the virus and promote trans-infection of neighbouring T lymphocyte[27]. In addition, HIV causes lymphoid tissue fibrosis through several mechanisms, including upregulation of T regulatory cells and release of transforming growth factor-β. Much of the harm associated with the virus in both untreated and treated disease probably occurs in these lymphoid structures[28,29].



The global prevalence of HIV has increased from 31 million in 2002, to 35.3 million in 2012, because people on antiretroviral therapy are living longer[30], whereas global incidence has decreased from 3.3 million in 2002, to 2.3 million in 2012[31]. Global AIDS-related deaths peaked at 2.3 million in 2005 and decreased to 1.6 million by 2012.

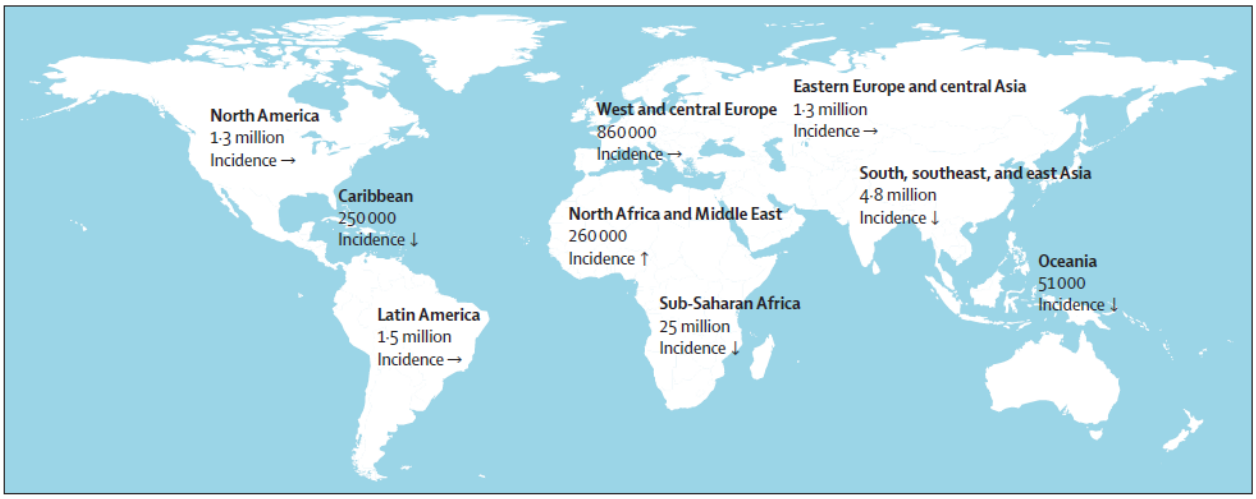


***Figure 1*: Estimated number of people living with HIV in 2012 and trends in the incidence of new infections from 2001 to 2012 by global region**
Data from UNAIDS 2013 report.[3]

1.2.2 HIV transmission

HIV is transmitted through contact of infected body fluids with mucosal tissue, blood or broken skin. The most important factor that increases the risk of sexual transmission of HIV-1 is the number of copies per mL of plasma HIV-1 RNA (viral load), with a 2.4 times increased risk of sexual transmission for every 1 log10 increase[32]. Acute HIV infection, which causes very high plasma viral loads in the first few months, is an important driver of HIV epidemics[33]. A reduction in plasma viral load of 0·7 log10 is estimated to reduce HIV-1 transmission by 50%[34]. Seminal and endocervical viral load independently predict risk of HIV-1 sexual transmission, after adjustment for plasma viral load[35]. Other factors associated with increased risk of sexual transmission of HIV include sexually transmitted infections (notably genital ulcers of any cause, herpes simplex type-2 infection, and bacterial vaginosis), pregnancy, and receptive anal intercourse[36-40]. Male circumcision is associated with a reduced risk of sexual transmission of HIV[41]. Behavioural factors that increase HIV-1 sexual transmission include many sexual partners and concurrent partnerships[42,43]. Findings of a study of African heterosexual serodiscordant couples showed that self-reported condom use reduced the per-coital act risk of HIV-1 transmission by 78%[44]. Injection and non-injection drug use, including alcohol, are associated with increased sexual risk behaviour, whereas injection drug use causes HIV transmission by shared needles[45]. Women who reported intimate partner violence had an increased incidence of HIV infection in a South African study[46].

1.2.3 Acute HIV infection

The detection of virus in the blood (typically measured as viral RNA levels) is often associated with a short symptomatic phase marked by fever, generalized lymphadenopathy, a nonspecific rash,

myalgias and/or malaise. More-severe complications — including meningitis — can occur, but many people are asymptomatic. The diagnosis of HIV-1 infection is based on the detection of specific antibodies, antigens, or both, and many commercial kits are available. Serological tests are generally used for screening. A major advance has been the availability of rapid HIV-1 antibody tests. For staging purposes, measurement of CD4+ cells and viraemia is required. Plasma viral load is widely used to monitor therapeutic success on antiretroviral treatment. Several commercially available tests provide sensitive quantification of plasma HIV-1 RNA copies.

During this period of primary or acute infection, the plasma levels of HIV RNA are typically at their peak (approximately 106–107 copies per ml). The severity of symptoms is strongly correlated with peak viral load during this phase of the infection[47]. Once the immune response develops, the levels of virus decrease by about 100-fold to a steady-state level that is often referred to as the viral set point[48]. This level can range from very few copies per ml of blood to approximately 106 copies per ml and tends to be higher in infants than adults. Importantly, the set point level is correlated with clinical outcome: individuals with high viral load set point typically progress more rapidly to AIDS and death than those with lower viral set point levels. Those very few individuals with very low viral load set points are referred to as HIV elite controllers and are of high interest.

1.2.4 Latent HIV infection

Through mechanisms yet poorly understood, HIV establishes quiescent (or latent) infection within memory CD4+ T cells. These cells are maintained indefinitely through homeostatic proliferation; some cells have stem-cell-like capacity for self-renewal. HIV can also establish long-term infection of naive CD4+ T cells, cells of the monocyte and macrophage lineage, and perhaps other long-lived cells[49-50].

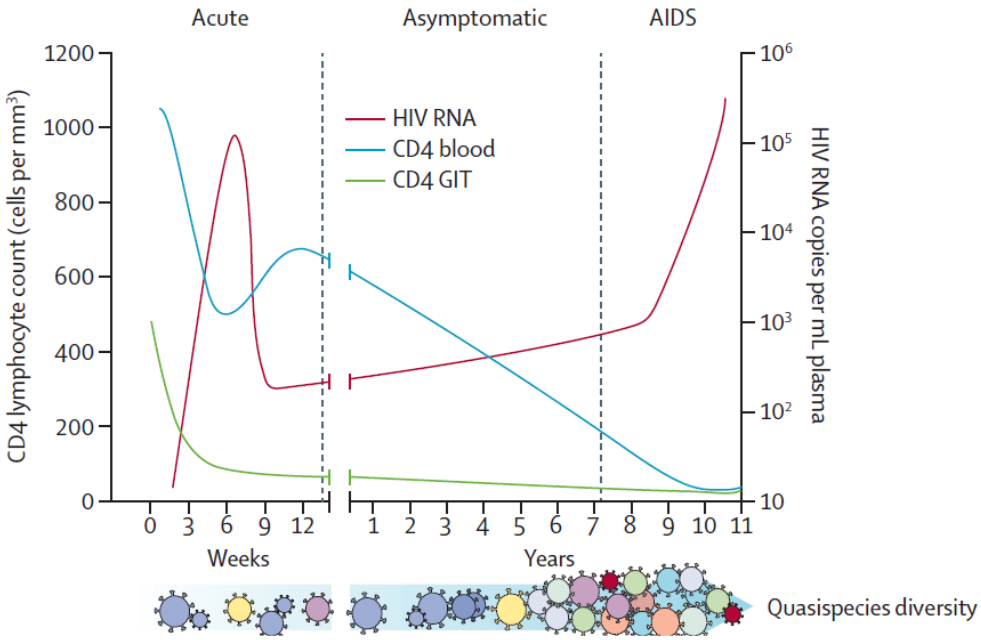
The hallmark of HIV infection is the progressive depletion of CD4 T cells because of reduced production and increased destruction. CD4 T cells are eliminated by direct infection, and bystander effects of syncitia formation, immune activation, proliferation, and senescence. In early infection, a transient reduction in circulating CD4 T cells is followed by recovery to near normal concentrations, which then slowly decrease by about 50–100 cells per μL. In addition to loss of total CD4 T cells, profound changes in T-cell subsets happen, including preferential loss of T-helper-17 cells and mucosal-associated invariant T cells, which are crucial for defence against bacteria[51].

HIV infection is also characterized by a marked increase in immune activation, driven by the direct effects of HIV as a ligand for the Toll-like receptor (TLR7 and TLR 8) expressed on plasmacytoid dendritic cells, leading to production of interferon-α[52]; microbial translocation, with lipopolysaccharide as a potent activator or TLR4 leading to the production of proinflammatory

cytokines such as interleukin 6 and tumour necrosis factor α (TNFα)[53]; co-infection with viruses such as cytomegalovirus that induce profound expansion of activated cytomegalovirus-specific T cells[54].

Much of the HIV replication and presumably CD4+ T cell death occurs in gut-associated lymphoid tissue, which harbours high numbers of susceptible memory T lymphocytes. The high levels of replication in gut-associated lymphoid tissue in primary infection causes severe T cell depletion that is thought to make the intestinal lining permeable; systemic translocation of bacterial products leads to increased immune activation[55]. HIV infection causes dramatic and sustained increases in the frequency of activated and proliferating CD4+ and CD8+ T cells, many of which are destined to die even in the absence of infection. HIV can also infect the thymus, leading to accelerated thymic loss (beyond what is observed with normal ageing), which contributes to the regenerative failure of T cells[56]. In the secondary lymph nodes, rapid loss of infected CD4+ T cells also occurs. The immunodeficiency that emerges as a result of this T cell depletion results in excess pathogen load and chronic inflammation, which in turn exacerbates harm to the lymphoid system by promoting, for example, tissue fibrosis in the lymph nodes[28]. The chronic inflammatory process stimulates immunoregulatory responses that blunt T cell function and might also impair haematopoiesis directly or indirectly, although the mechanisms underlying the effects on the bone marrow are unclear[57,58].

This generalized activation of the immune system contributes to the progressive loss of these cells, and the degree to which HIV infection causes T cell activation is an independent predictor of the rate at which individuals lose CD4+ T cells and progress to the Acquired Immuno-Deficiency Syndrome (AIDS)[59.60].



Natural history of untreated HIV infection

1.2.5 HIV infection staging

A confirmed case can be classified in one of five HIV infection stages (0, 1, 2, 3, or unknown)[61]. If there was a negative HIV test within 6 months of the first HIV infection diagnosis, the stage is 0, and remains 0 until 6 months after diagnosis. Otherwise, the stage is determined by the CD4 test immunologic criteria shown in the following table:

	Age on date of CD4 T-lymphocyte test					
	<1 year		1—5 years		6 years through adult	
Stage*	Cells/µL	%	Cells/µL	%	Cells/µL	%
1	≥1,500	≥34	≥1,000	≥30	≥500	≥26
2	750—1,499	26—33	500—999	22—29	200—499	14—25
3	<750	<26	<500	<22	<200	<14

If a stage-3-defining opportunistic illness has been diagnosed, the stage is 3 (AIDS).

Stage-3-Defining Opportunistic Illnesses in HIV Infection:

Bacterial infections, multiple or recurrent; Candidiasis of bronchi, trachea, or lungs; Candidiasis of esophagus; Cervical cancer, invasive; Coccidioidomycosis, disseminated or extrapulmonary; Cryptococcosis, extrapulmonary; Cryptosporidiosis, chronic intestinal (>1 month's duration); Cytomegalovirus disease (other than liver, spleen, or nodes), onset at age >1 month; Cytomegalovirus retinitis (with loss of vision); Encephalopathy attributed to HIV; Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis (onset at age >1 month); Histoplasmosis, disseminated or extrapulmonary; Isosporiasis, chronic intestinal (>1 month's duration); Kaposi sarcoma; Lymphoma, Burkitt (or equivalent term); Lymphoma, immunoblastic (or equivalent term); Lymphoma, primary, of brain; Mycobacterium avium complex or Mycobacterium kansasii, disseminated or extrapulmonary; Mycobacterium tuberculosis of any site, pulmonary†, disseminated, or extrapulmonary; Mycobacterium, other species or unidentified species, disseminated or extrapulmonary; Pneumocystis jirovecii (previously known as "Pneumocystis carinii") pneumonia; Pneumonia, recurrent; Progressive multifocal leukoencephalopathy; Salmonella septicemia, recurrent; Toxoplasmosis of brain, onset at age >1 month; Wasting syndrome attributed to HIV.

1.2.5 HIV infection treatment options

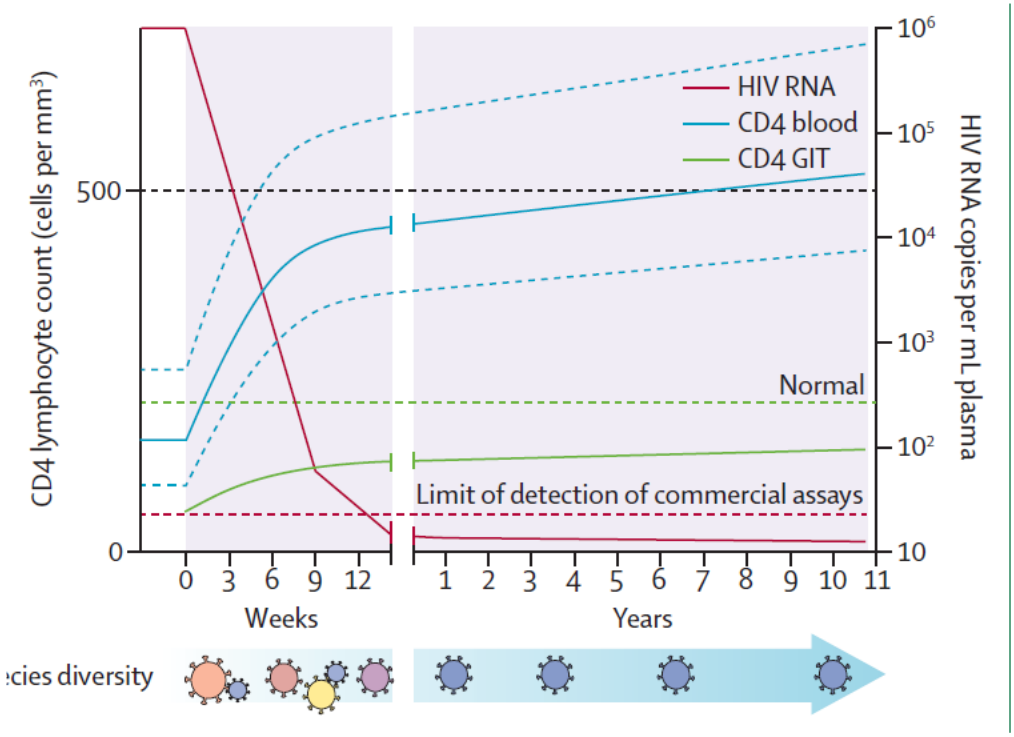
The development of combination antiretroviral therapy (ART) is often touted as one of the greatest achievements of modern medicine. When given to adherent and motivated individuals, contemporary combination regimens reduce the level of viraemia by several orders of magnitude in a matter of weeks. The degree of viral suppression is so great that viral evolution and the emergence of drug-resistant mutations are prevented. In principle, these regimens should work indefinitely. In the absence of viral replication, the immune system recovers much of its lost function and AIDS is prevented.

Approximately 25 unique antiretroviral drugs have been approved for use in adults by the US and European regulatory agencies. These drugs span five therapeutic classes, each targeting a unique step in the life cycle of the virus[62].

Drug Class	Mechanism of Action	First Approved Drug	Approval Date
Nucleoside reverse transcriptase inhibitors	Inhibit reverse transcriptase via chain termination	Zidovudine	March 19, 1987
Nonnucleoside reverse transcriptase inhibitors	Inhibit reverse transcriptase via direct binding and inactivation	Nevirapine	June 21, 1996
Protease inhibitors	Inhibit HIV protease, an enzyme necessary for catalytic cleavage of proteins needed for viral replication	Saquinavir	December 6, 1995
Fusion inhibitors	Block entrance of HIV into CD4 cells	Enfuvirtide	March 13, 2003
CCR5 antagonists	Block CCR5 receptors on CD4 cell surfaces, preventing HIV entrance	Maraviroc	August 6, 2007
Integrase inhibitors	Inhibit integrase, an enzyme necessary for integration of viral DNA into host cells	Raltegravir	October 12, 2007

The optimal time to start ART has been debated for many years. Some expert panels have argued that as AIDS event rates are low in people with high CD4+ T cell counts, the potential toxicities associated with ART could outweigh any benefit and therapy should be deferred[63]. Other groups have argued that ART should be recommended in early-stage disease because it prevents irreversible harm to the immune system and perhaps other organ systems, improves qualify of life and reduces the risk of transmission[64]. ART is expected to be recommended for anyone with HIV infection. However, individuals who are not ready to commit to therapy should not be treated until they feel that they can adhere to therapy (to avoid promoting virus drug resistance), or until CD4+ T cell counts decline to levels that make waiting too risky in terms of developing AIDS-related complications. Regardless of disease state, ART is generally recommended in HIV-discordant

couples, HIV-positive women who are pregnant or who are breastfeeding, individuals co-infected with hepatitis B and in the WHO guidelines for all HIV-positive children under 5 years of age[65].



Natural history of HIV infection under antiretroviral therapy

1.3 CO-RELATION BETWEEN HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND PRE-INVASIVE AND INVASIVE CERVICAL CANCER

1.3.1 The interplay between HPV and HIV infection

HIV-related immunodeficiency has complex effects on female genital HPV, which include increased risks of infection, multiple types, persistence, reactivation and the risk to develop pre-invasive and invasive disease. Women with HIV infection are more likely to develop pre-neoplastic and neoplastic cervical lesions caused by HPV when compared to seronegative women[66]. It is estimated that the prevalence of this disease is three times higher in this target group, especially in view of the decrease of CD4+ T lymphocytes and the higher levels of viral load[67]. Cervical cancer is an AIDS-defining illness, but the relative risk for developing cervical cancer in HIV-positive women compared with HIV-negative women varies widely across the globe. In developed countries where the incidence of cervical cancer is relatively low in the general population, the relative risk of cervical cancer among HIV-positive women is relatively high, particularly among those groups of women in these countries with poor access to routine medical care. In the US and in southern Europe, these tend to be women with a history of injection drug use. In the developing world, where cervical cancer is more common among HIV-negative women than in the developed world, the relative risk among HIV-positive women is lower than in the developed world, although the absolute incidence is higher. Another factor that may be depressing the relative risk among HIV-positive women in developing countries is competing mortality from other HIV-associated illnesses since development of cervical cancer may take decades. Many studies have shown that HIV positivity is associated with increased prevalence of cervical HPV infection and CIN, and the risk primarily resides with increased HPV persistence, which in turn may reflect HIV-1- or -2-related immunosuppression[68.69]. Prospective cohort studies of HIV-positive women often reveal detection of additional HPV types in each woman over time, particularly among those with lower CD4 levels. Careful analysis of sexual behaviors indicates that a substantial proportion of newly detected HPV infections may represent reactivation of previously acquired infections rather than recent sexual transmission[70].

Analysis of the relationship between declining CD4 level and specific HPV types also reveals differences in the effects of host immune status on the risk of HPV infection. HPV16, the most common HPV type in cervical cancers, was the least affected of all HPV types by diminished immunity as measured by CD4 T cell level, largely because it was detected at relatively high levels even among women with high CD4 levels[71]. In contrast, other HPV types were less common at higher CD4 levels and showed a proportionately larger increase with lower CD4 levels. These

observations suggest that HPV16 may more efficiently evade immune surveillance than the other HPV types and may in part explain why HPV16 is the predominant HPV type in cervical cancers, including those of otherwise healthy, immunocompetent women. It follows that HPV types other than HPV16 may account for a higher proportion of cervical cancers among more immunosuppressed HIV-positive women – a hypothesis that is as yet untested. If confirmed, however, these results have implications for the efficacy of HPV vaccines to prevent cervical cancer in HIV-positive women, since the HPV vaccines that are furthest along in development contain only two oncogenic types (HPV16 and HPV18)[72].

In a study involving 1778 HIV-positive and 500 HIV-negative women, it was found that 63% of the HIV-positive participants tested positive to HPV viral DNA while only 30% of the HIV-negative participants tested positive[73].

A recent meta-analysis investigated the studies evaluating the risk of HPV related pre-invasive and invasive cervical cancer in sub-Saharan Africa[74]. The meta-analysis showed that HIV positivity is associated with increased prevalence of HPV infection on different anatomic sites. In particular, according to Bosch et al., HIV-positive women had two- to 22-folds increase risk of developing cervical cancer as compared to HIV-negative women[75]. Two studies demonstrated a 2.4 standard incidence ratio (SIR) of cervical cancer in HIV-positive women from Uganda AIDS–Cancer Registry Match Study[76], and the second from Senegal showed a substantial increase in the risk of invasive cervical cancer (ICC) which was observed with OR of 6.5 for ICC (95% CI 2.1–19.8) in HIV-infected women compared with the control group of HIV-negative women[77]. The incidence of cervical cancer in a linkage of 15,000 HIV-infected individuals from Kyadondo County in Uganda reported 70 per 100,000 women-years and an SIR of 2.7 among HIV-infected women as compared to HIV-uninfected women[78]. Another study in Nigeria reported a two-fold higher risk of cervical cancer in people living with HIV/AIDS[79]. Two recent reviews have shown that HIV positivity is associated with increased prevalence of cervical HPV infection and cervical intraepithelial neoplasia (CIN)[72,80].

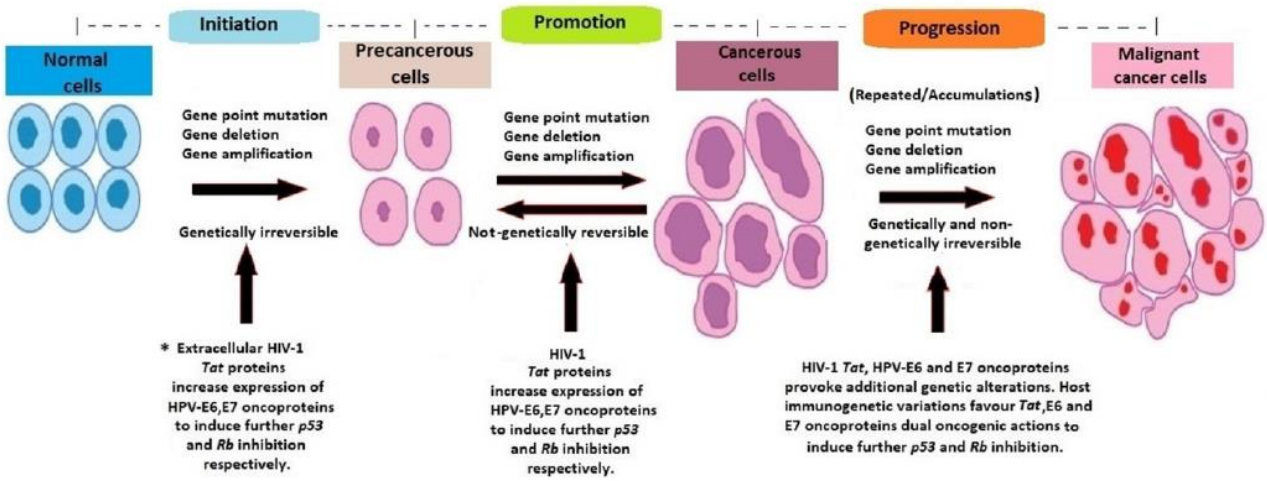
In contrast to other HIV-associated malignancies, the incidence of HPV-associated cancers such as anal cancer has increased, not decreased since the introduction of ART[81]. Others have shown a benefit for clearance of cervical HPV infection in HIV-infected women and anal HPV infection in men upon initiation of ART but the prevalence of both cervical and anal HPV infection remains high among HIV-infected individuals in the ART era[82]. Development of HPV-associated neoplasia is initiated upon HPV entry into basal and parabasal cells of the epithelium. Animal model work suggests that HPV penetration through multiple layers of the stratified squamous epithelium requires a mechanical breach or tear[83]. It is possible that co-infection with viruses such as HIV may also lead

to epithelial disruption. If so, HIV-associated epithelial disruption may be one mechanism contributing to the increased risk of HPV infection among HIV-infected individuals.

Tat and gp120 are HIV proteins that are secreted from HIV-infected intraepithelial immune cells. These proteins may play an important role in disruption of epithelial tight junctions (TJ) and HPV entry into epithelium. Tat is a transactivator protein that activates integrin and mitogen-activated protein kinases (MAPK) signaling, which in turn may disrupt endothelial and epithelial cell junctions through aberrant internalization of TJ proteins[84,85]. HIV gp120 is an envelope protein that binds to galactosyl ceramide of epithelial cells, induces intracellular calcium elevation and activates protein kinase C (PKC). PKC activates p38 mitogen-activated protein kinases, phosphoinositide 3 kinases/protein kinase B (PI3K/AKT), and c-Jun N-terminal kinases (JNK). This leads to the disruption of epithelial TJ through modulation of TJ protein internalization and/or TJ gene expression. If HIV infection is indeed a biologically relevant contributor to mucosal epithelium disruption and entry of HPV into epithelium, then HIV proteins should be present in the mucosal environment, even among HIV-infected individuals with well-controlled HIV viral load on ART. Cell-free HIV-1 virions and viral DNA/RNA can be isolated from oral and genital mucosal epithelium, as well as the saliva and cervicovaginal secretions of HIV-infected individuals. HIV-infected lymphocytes, and Langerhans cells can be detected in the mucosal and submucosal layers of oral and genital epithelium and HIV virions can be detected by electron microscopy, within the TJs of oral epithelium. Finally, replicating HIV and HIV-infected cells have been found within cervical epithelia of HIV-infected women, including women on ART.

In some cases, HIV/HPV-associated cervical cancerous cells, non-genetically regress spontaneously to pre-cancerous cells. Persistent accumulation of uncorrected mutations and additional pro-oncogenic effects of HIV-1 Tat and HPV E6, E7 oncoproteins, lead to the progression of cancerous cells to invasive malignant cancer cells, regardless of the use of ART. The immunosuppressive effects of HIV infection are associated with the rapid progression of HPV-induced cervical pre-malignant lesions. This may influence the rapid onset of cervical disease and further effects on clinical outcome.

HIV proteins can directly cause cancer growth by interfering with cellular functions. For example, HIV Tat proteins directly interact with the host tumor suppressor genes p53/pRb/p130/p107 and induce increased cell proliferation, which promote the effect of HPV oncoproteins E6 and E7 in the rapidly progressing cervical carcinogenesis. The increased rate of HPV-associated cervical disease in HIV positive women is aggravated by HIV/HPV molecular interactions because HIV Tat proteins can modulate HPV E2 gene expression, which in turn, influences HPV viral replication[86].



Host molecular genetic variations and alterations in HIV/HPV co-infected cervical carcinogenesis

1.4 NEUROTROPHINS AS A POSSIBLE LINK BETWEEN HIV INFECTION AND UTERINE CERVIX HPV-RELATED ONCOGENESIS

1.4.1 Neurotrophin family and their role in the neuronal development and function

Neurotrophins are a family of proteins that induce the survival, development, and function of neurons[87,88]. Nerve growth factor (NGF), the first such factor to be characterized, was discovered during a search for such survival factors[89]. There are four neurotrophins characterized in mammals. NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) are derived from a common ancestral gene, are similar in sequence and structure, and are therefore collectively named neurotrophins[90]. Neurotrophins generally function as noncovalently associated homodimers, but at least some neurotrophin subunits are able to form heterodimers with other neurotrophin subunits. Initial efforts to identify NGF receptors resulted in discovery of a receptor now named p75NTR, is a distant member of the tumor necrosis factor receptor family[91]. For many years this was believed to be a low-affinity receptor specific for NGF. More recently, it has been shown to bind to all of the neurotrophins with a very similar affinity[92]. Subsequently, the three members of the Trk (tropomyosin-related kinase) receptor tyrosine kinase family were shown to be a second class of neurotrophin receptors[93]. The neurotrophins have been shown to directly bind and dimerize these receptors, which results in activation of the tyrosine kinases present in their cytoplasmic domains. NGF is specific for TrkA. BDNF and NT-4 are specific for TrkB. NT-3 activates TrkC and is also able to activate less efficiently each of the other Trk receptors.

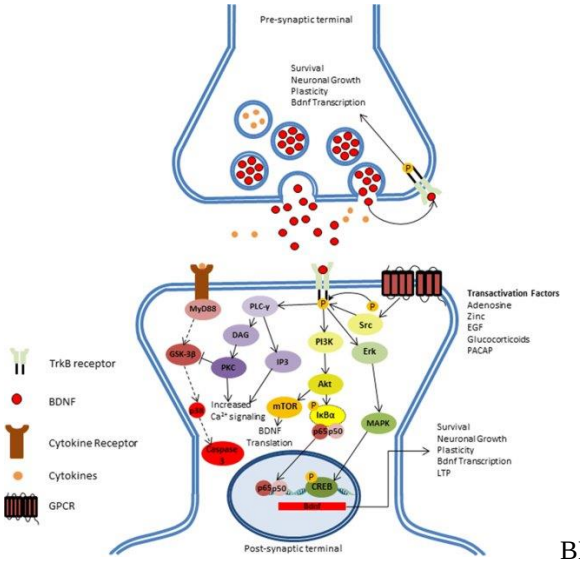
Tyrosine kinase–mediated signaling by endogenous Trk receptors appears to promote survival and/or differentiation in all neuronal populations examined to date. Ligand engagement of Trk receptors has been shown to result in phosphorylation of cytoplasmic tyrosine residues on the cytoplasmic domains of these receptors. Trk receptors binding causes to intracellular signaling cascades, which include the Ras/ERK (extracellular signal–regulated kinase) protein kinase pathway, the phosphatidylinositol-3-kinase (PI-3 kinase)/Akt kinase pathway, and phospholipase C (PLC)-γ1[94].

In cell culture, many populations of central nervous system (CNS) precursors are regulated by neurotrophins. Nestin-positive cells from the rat striatum can be induced to proliferate with NGF[95]. The proliferation and survival of oligodendrocyte precursors (O2A progenitors) have been shown to be promoted by NT-3 in vitro and in vivo[96]. In other instances, neurotrophin application has been shown to induce differentiation of precursors. For example, NT-3, but not other neurotrophins, promotes differentiation of rodent cortical precursor[97]. The differentiation of hippocampal neuron precursors is promoted by BDNF, NT-3, and NT-4[98]. Although these observations suggest that

neurotrophins regulate the size of precursor pools and neurogenesis in vivo, analyses of the various neurotrophin and Trk receptor knockouts has provided no evidence that development of these cell populations within the CNS is perturbed during embryogenesis.

The signaling pathways activated by neurotrophins through Trk receptors result in many neuronal functions, such as cell survival, differentiation, dendritic arborization, synapse formation, plasticity, axonal growth, and axonal guidance. Functions ascribed to the p75NTR receptor are diverse, complex, and sometimes contradictory. p75NTR has been implicated in both promoting survival and inducing apoptosis, enhancing neurite outgrowth and facilitating growth-cone collapse, and mediating differentiation and enhancing proliferation. Moreover, p75NTR may also play a role in myelination.

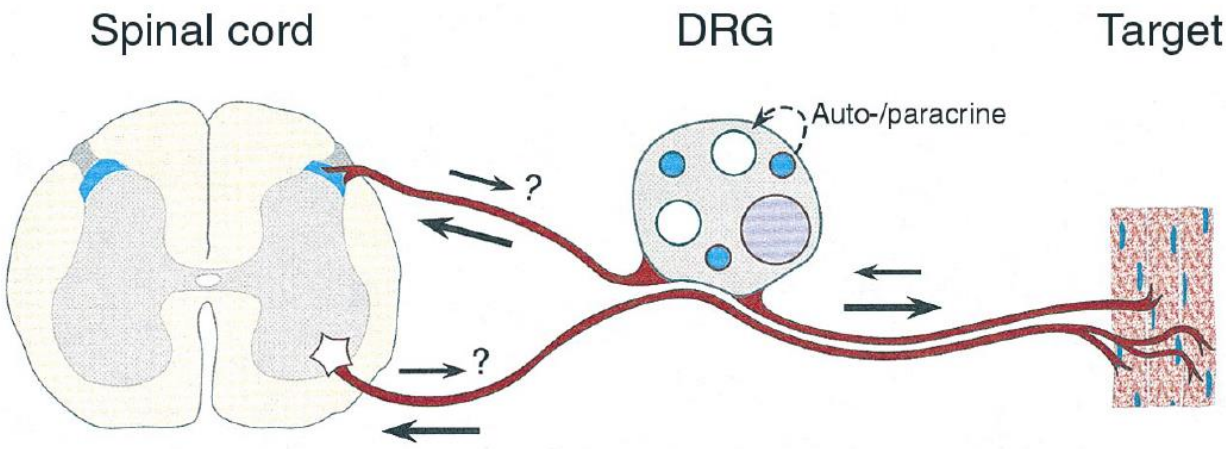
In physiological conditions, binding of BDNF to TrkB receptor in either paracrine or autocrine signaling elicits three distinct downstream pathways. BDNF-dependent phospholipase C-gamma (PLC-γ) can induce short-term signaling by increasing Ca2+ neuronal response and inhibit inflammatory-dependent apoptosis cascade by inhibition of glycogen synthase kinase 3-beta (GSK-3β). Induction of phosphatidylinositol 3-Phosphate (PI3K) induces transcription of BDNF mRNA by activating mTOR-dependent translation of BDNF. Additionally, BDNF can modulate gene regulation by activating NF-κB and CREB transcription factors by inducing Akt and Erk downstream pathways, respectively. Gene modulation induces neuronal survival, growth, long-term potentiation (LTP), and de novo expression of BDNF. In addition, BDNF-independent transactivation of TrkB can also play an important role in the neurotrophic pathway regulation by factors, such as adenosine, zinc, epidermal growth factor (EGF), glucocorticoids, and pituitary adenylate-cyclase-activating polypeptide (PACAP), further enhancing TrkB signaling in the synapse[99].



BDNF induces survival-related signaling mechanisms

1.4.2 Neurotrophin anterograde transport

The traditional role of neurotrophins as retrogradely transported, target-derived survival factors is well established in the developing peripheral nervous system (PNS). NGF is produced by targets of NGF-dependent neurons in limiting quantities and promotes neuron survival through its retrograde transport and signaling to the cell body. Retrograde transport was first demonstrated with injections of 125I-labeled NGF into the terminal fields of sympathetic and sensory neurons in the PNS or cholinergic neurons in the CNS[101,102]. Intracranial and intranerve injections of iodinated BDNF, NT-3 and NT-4 result in their retrograde transport to adult PNS and CNS neuron cell bodies[103].
Initially, BDNF expression was thought to be restricted to the CNS[104]. Authors showed that sub-populations of sensory neurons are BDNF responsive during development[105,106]. The presence of the NT-3-binding molecules TrkC, TrkB and p75 in the adult optic tectum, and their presence on developing tectal cells whose survival required an anterogradely transported signal, prompted von Bartheld to inject 125I-labeled NT-3 into the chicken embryo eye and look for evidence of anterograde transport. Indeed, 125I-NT-3 accumulated in the optic tectum, where it was associated with presynaptic vesicles[107]. Recent studies have shown that BDNF is normally concentrated in the superficial layers of the dorsal spinal cord. Thus, BDNF appears to be packaged much like a neurotransmitter in central processes of dorsal root ganglion (DRG) neurons[108]. It is important to note that, in all these studies, there is no anterograde accumulation of NGF, and that the accumulation of NT-3 is much less than that of BDNF. Anterogradely trafficked BDNF might have trophic actions on targets of BDNF-expressing neurons or on neighboring terminals of non-BDNF-expressing neurons.



Based on the properties of anterograde transport, there are multiple roles for the BDNF that is anterogradely trafficked by BDNF-expressing central and peripheral neurons: (1) a neurotransmitter-like role for rapid postsynaptic functions; (2) a source of BDNF to astrocytes, particularly in injured brain; (3) as a trophic agent for postsynaptic targets such as skin, muscle, or gut; and (4) as a signaling molecule between nerve terminals of homologous neurons (e.g. nigrostriatal dopamine neurons) or heterologous neurons (e.g. release from corticostriatal terminals and binding by nigrostriatal terminals), which can then internalize and retrogradely transport the protein.

1.4.3 Neurotrophins expression in the viscera epithelia

There is good evidence for the fundamental role of target-derived BDNF for the development of visceral innervation and it has been observed that inflammatory diseases of the adult viscera are associated with a strong increase in local BDNF mRNA and protein production[109-111].

Lommatzsch et al. systematically investigated the expression and potential role of BDNF in the targets of adult visceral sensory and motor neurons. Using a nonradioactive in situ hybridization technique, authors identified the cells synthesizing BDNF mRNA in all gastrointestinal regions and in tissues of the cardiorespiratory and urogenital systems. In addition, they quantified the amounts of BDNF protein present in these internal organs and examined the distribution of BDNF receptors and the morphology of viscera in mice lacking BDNF[112]. Authors found that BDNF is expressed in certain viscera in even higher amounts than in the brain. In particular, BDNF protein levels in the kidney were comparable to those found in the brain. In contrast, lysates taken from the urinary bladder revealed a much higher concentration of BDNF. Proximal and distal tubules of the kidney displayed strong BDNF expression. No expression was observed in glomerula (Figure 2, kidney). Renal vascular smooth muscle cells appeared to be BDNF mRNA negative (not shown). Urothelia of the urinary bladder revealed very strong BDNF expression. In contrast, the tunica muscularis was BDNF mRNA-negative (Figure 3, bladder). BDNF mRNA was found in epithelia of the uterus and oviduct. There was also a light expression in adjacent cells of the lamina propria (tuba uterina). Interestingly, the uterus showed a unique receptor pattern characterized by an expression of trkB in the squamous epithelium of the portio and p75NTR in smooth muscle and connective tissue cells. Strikingly, these structures appeared BDNF mRNA-negative. Thus, non-neuronal cells of the viscera showed a reciprocal expression pattern of either BDNF or trkB or p75NTR.

The observed p75NTR-IR on the myometrium could indicate the plasticity and growth potency of the uterine smooth muscle. Autocrine processes of BDNF seem not to be involved, because the

myometrium was completely negative for BDNF mRNA. Paracrine actions of BDNF, however, have to be considered not only on uterine smooth muscle cells, but also on the squamous epithelium of the portio, because BDNF has been demonstrated recently to promote keratinocyte proliferation via trkB[113].

Organ	Compartment	BDNF mRNA	trkB-IR	$p75^{NTR}$-IR
Submand. gland	acini	+	–	–
	ducts	–	–	–
Sublingual gland	acini and ducts	–	–	–
Esophagus	squamous epithelium	+	–	–
	tunica muscularis	+/–	+/–	–
	myenteric plexus	++	++	++
Stomach	foveolae gastricae	+	–	–
	gastric glands	++	–	–
	tunica muscularis	+/–	–	–
	myenteric plexus	++	++	++
Duodenum	epithelium	+++	–	–
	tunica muscularis	+/–	–	–
	myenteric plexus	++	++	++
Ileum	epithelium	+	–	–
	tunica muscularis	+/–	–	–
	myenteric plexus	++	++	++
Colon	epithelium	+++	–	–
	tunica muscularis	+/–	–	–
	myenteric plexus	++	++	++
Lung	respiratory epithelium	+++	–	–
	airway smooth muscle	++	–	–
	blood vessels	++	–	–
Heart	cardiomyocytes	++/–	–	–
Liver	hepatocytes	++	–	–
	portal triad	–	–	–
Pancreas	exocrine glands	+++	–	–
	exocrine ducts	–	–	–
	islet cells	+	–	–
Kidney	tubules	+++	–	–
	thin segments	+	–	–
	glomerula	–	–	–
Oviduct	columnar epithelium	++	–	–
	lamina propria	+	–	+++
	tunica muscularis	–	–	++
Uterus	columnar epithelium	++	–	–
	lamina propria	+	–	+++
	myometrium	–	–	++
Portio vaginalis	squamous epithelium	–	++	–
Bladder	urothelium	+++	–	–
	tunica muscularis	–	–	–

BDNF Expression and BDNF Receptors in Internal Organs

1.4.4 Role of neurotrophins in gynecological cancers

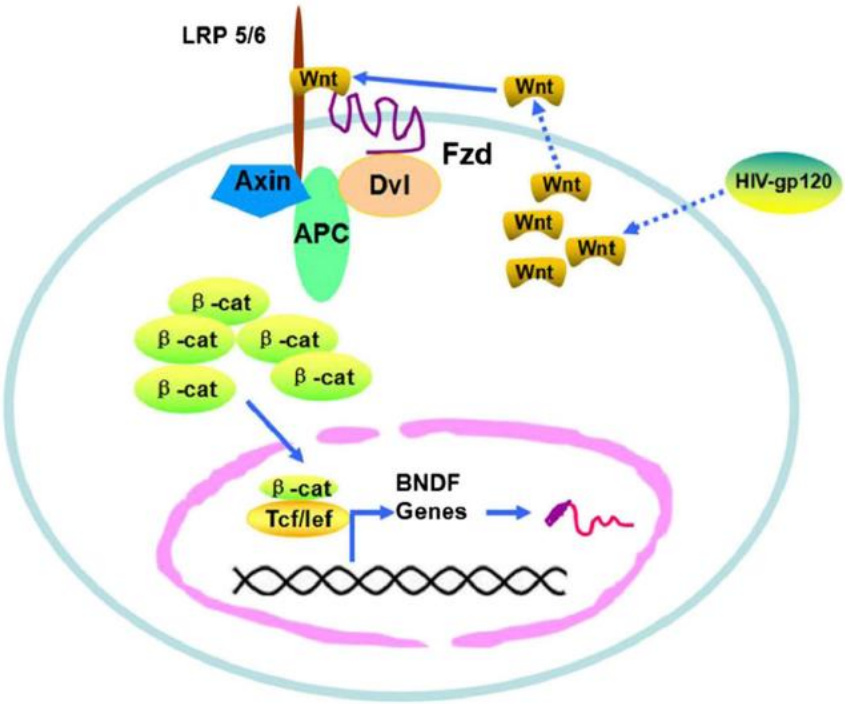
During the neoplastic processes, neurotrophins and their receptors are overexpressed by tumoral cells, promoting progression and angiogenesis in several cancer models. For instance, the expression of neurotrophins predicts poor survival rates in breast and ovarian cancer patient and neurotrophins have been proposed as potential therapeutic targets in these neoplasms[114-116].

In particular, one study performed in matrigel plugs in immunedeficient mice shows that NGF strongly increases invasion, cord formation and the monolayer permeability of endothelial cells[117]. Furthermore, a recent work shows that NGF increases mcell proliferation, migration and differentiation of the human endothelial cell line EA.hy926 in a dose-dependent manner[118]. In the same way, BDNF increases angiogenic tube formation of the endothelial cells in HUVEC and the overexpression of BDNF in a mouse endothelial cell line promotes endothelial cell proliferation, migration, invasion, and survival. This evidence indicates that BDNF/TRKB exhibits a direct role in the angiogenic process[119,120]. On the other hand, both NGF and BDNF have an indirect angiogenic role, mediated by VEGF modulation in different cellular models. It is described that NGF and BDNF induce VEGF expression in MAPK/ERK 2-dependent pathways in granulosa cells and osteoblasts, respectively[121,122]. Besides, NGF promotes VEGF expression in neuronal superior cervical ganglia, while BDNF increases VEGF expression in human chondrosarcoma and neuroblastoma cells[123-125]. Another key point is that plasmatic levels of VEGF are lower in deficient BDNF animals compared to wild type animals[126]. All these antecedents indicate that NTs not only act directly in vascular cells, but also affect several cell types by increasing VEGF expression and therefore their angiogenesis potential.

It has been described that BDNF and TRKB expression are significantly higher in cervical cancer tissues than in normal tissues and that their presence is higher in advanced stages of this neoplasm. In addition, BDNF levels are positively associated with lymph node metastasis in cervical cancer patients[127,128]. In cervical cancer cell lines, BDNF/TRKB increases cell proliferation apparently involving ERK and AKT signaling pathways[129,130]. Considering that the activation of ERK signaling pathway by BDNF/TRKB was associated with an increase of VEGF expression in osteoblasts, and given that TRKB can activate PI3K and ERK signaling pathways which regulate VEGF expression in several models, it is plausible that the VEGF expression could be increased by TRKB in cervical cancer[131].

1.4.5 HIV infection modulates neurotrophins expression

Considerable experimental data indicate that the HIV-1 exterior envelope glycoprotein gp120 (HIV-1 gp120), which is shed from the virus, can cause neurotoxicity by multiple mechanisms. A recent study found that HIV-1 gp120 in post-mortem tissues of the spinal cord dorsal horn was significantly higher in the pain-positive HIV-1 patients, compared to the pain-negative HIV-1 patients[132]. Additionally, intrathecal injection of HIV-1 gp120 developed mechanical allodynia and visceral hyperalgesia in rodents. These findings suggest that gp120 may critically contribute to the pathogenesis of HIV-associated pain. Wang et al. demonstrated, in microglial cell line BV2 cells, gp120 stimulation upregulated BDNF expression[133]. Based on the proposed role of microglial BDNF in pain pathogenesis[134], authors suggested that gp120 stimulated BDNF expression may contribute to the development of HIV-associated pain. In the proposed model, Wang et al. suggested that gp120 stimulates BV2 to up-regulate Wnt ligands (e.g., Wnt3a). The stimulated BV2 cells then secrete Wnt protein. The secreted Wnt ligands bind to Frizzled receptors and LRP5/6 co-receptors and cause the stabilization and nuclear translocation of β-catenin. Once in the nucleus, β-catenin binds to the BDNF promoter to activate the transcription.



HIV-1 gp120 up-regulates BDNF expression in BV2 cells via Wnt/β-catenin signaling pathway

Furthermore, in a recent study Zhou et al. found that Wnt3a and β-catenin expression levels increased in the spinal cord of HNP mice, consequently regulating the expression of BDNF and affecting

hypersensitivity. In addition, the blockade of Wing-Int/β-catenin signaling, BDNF/TrkB or the BDNF/p75NTR pathway alleviated mechanical allodynia.

Another study demonstrated that Tat, produced by macrophage HIV reservoirs in the brain, increased expression of C5, APBA1, and BDNF, and decreased CRLF2, causing the production of cytokines/chemokines and viral proteins that promote inflammation and neuronal damage, playing a key role in HIV neuropathogenesis[136]. Liu et al. showed that exposure to Tat reduced the length and number of dendrites in cultured neurons, downregulating CREB activity and CREB-mediated gene (BDNF, c-fos, Egr-1) expression[137]. On the other hand, some authors have demonstrated a protective role of BDNF against the neurocognitive impairment associated with HIV infection. Increased BDNF levels have been shown to decrease the odds of developing HIV-associated neurocognitive disorder and some published data show that BDNF reduces degeneration of synapses and axons triggered by viral proteins[138]. Therefore, BDNF has been shown to exert dose-dependent opposite effects in the CNS[139].

CHAPTER II

OBJECTIVES

Since published data show that neurotrophins expression, in particular BDNF, is upregulated in invasive cervical cancer and that neurotrophins are also upregulated in peripheral nervous system in patients with HIV infection, we hypothesize that neurotrophins, in HIV infected women, play a crucial role in the enhancement and in the accelerated progression of cervical pre-invasive and invasive lesions.

Therefore, the aim of this study is to evaluate the expression of neurotrophins NGF and BDNF in specimens from pre-invasive or invasive cervical cancer lesions according to the HIV status of the patients.

CHAPTER III

METHODS

Patients were prospectively recruited from the regional reference center for infectious diseases in obstetrics and gynecology at our gynecological department of the University of Naples "Federico II" from 2018 to 2019. Patients with following inclusion criteria were enrolled: age between 15 and 50 years old; BMI<30 kg/m^2; no other comorbidities apart from HIV infection; pre-invasive cervical cancer (CIN 2-3) or carcinoma in situ at histological diagnosis of specimens from LEEPs for suspected cervical cancer (abnormal pap-smear, abnormal colposcopy findings, abnormal histological findings at cervical biopsy). We collected clinical data: age, smoking status, parity, HIV infection stage, ART therapy, previous pap-smears, and HPV genotyping. All included women underwent a transvaginal ultrasound for the assessment of uterus, ovaries, and inguinal lymph nodes: only women with normal ultrasound findings were included in the study.

Indications for LEEP were grouped as follows: 1. Persistent L-SIL or ASCUS, ASC-H at citology; 2. H-SIL at citology; 3. CIN 2/3 at histology of cervical biopsies during colposcopy exam.

The study was conducted following the Declaration of Helsinki (1975) and Good Clinical Practice guidelines. Before enrolment, the purpose of the study was clearly explained, and all patients received detailed information about the study, to which they gave their consent. The information obtained were anonymized before analysis.

LEEP samples were sent for histological analysis already fixed in 10% formaldehyde and oriented by the gynecologist. Firstly, length, width and wall thickness were measured, for each LEEP sample; any grossly visible lesion was recorded in terms of length, width and thickness; lesion location and its distance from margins were documented. Then, the deep margin was inked and full sample is submitted to histologic examination. Specimens were thinly sectioned radially (ideally 2mm sections), ensuring each section has endocervical and ectocervical margins. They are serially submitted in a clockwise direction by putting one or two sections per cassette, depending on the size.

Tissue embedding and cassette processing

Because melted paraffin is hydrophobic, most of the water must be removed from tissues. Therefore, labeled cassettes were immersed in a series of ethanol of increasing concentration, from 70% to 100%: 15 minutes in 70% ethanol, 15 minutes in 90% ethanol, 15 minutes in 100% eth

anol, 15 minutes again in 100% ethanol, 30 minutes in 100% ethanol and, lastly, 45 minutes in 100% ethanol. Due to this process, all water is progressively replaced from alcohol. Before paraffin can infiltrate the tissue an intermediate solvent, miscible with both ethanol and paraffin is used. This step

is called clearing, because many intermediate agents give optical clarity to the tissue due to their high refractive index. Clearing stage for specimens no more than 4mm thick is carried out with three steps in xylene (20 minutes, 20 minutes, and 45 minutes respectively). At this point, tissues are infiltrated with paraffin wax in a three-step cycle (30 minutes, 30 minutes and 45 minutes). The process is vacuum induced (260mmHg) at constant temperature (60°C). When the specimen is fully infiltrated with paraffine wax, it is orientated and placed in a mold filled with molten wax using an embedding center (HistoCore Arcadia H, Leica BioSystems). The cassette is placed on top of the mold and put on a cold surface to solidify and form a block. T

Slide preparation and coloration

Block are cut with a microtome to make thin slices of tissue (4μm) which are floated over a water bath (5-9°C) and picked up on a slide (Mustafa Q. Yousif, Shadi A. Qasem, in Skin Tissue Engineering and Regenerative Medicine, 2016). Once slides are ready, they can be stained with Hematoxylin and Eosin (H&E). Sections are deparaffinated, slides flamed on burner and placed in xylene solution. After repetition of the previous passages, tissue sections are hydrated by passing through decreasing concentration of ethanol baths and water (100%, 90%, 80%, 70%). Sliced are stained in Hematoxylin for 3-5 minutes, washed in water, differentiated in a solution of 1% HCl in 70% ethanol for 5 minutes. After another wash in water, slides are stained with eosin for 10 minutes, rewashed and rehydrated in increasing concentrations of ethanol and xylene and mount in mounting media. After all these steps, all LEEP sections were evaluated under the microscope by a pathologist for diagnosis and choice of the most appropriate sections for further protein expression evaluation.

Neurotrophin expression in cervical dysplastic and neoplastic lesions

NGF and BDNF expression was evaluated using immunohistochemistry (IHC). Initially, one or two sections per patient were chosen among the others by considering morphological appearances at H&E staining: only section with dysplastic and neoplastic lesion of vulvar epithelium were included, and, for each patient, slides containing larger stromal portions were considered more suitable for the experiment so to have an internal positive control. Human brain tissue slides were used as control of the entire procedure and for specific protocol suiting. Immunohistochemistry was executed with Benchmark Ultra Roche Ventana automated system. Samples were deparaffinated at 72°C. Heat induced epitope retrieval was performed at 100°C for 54 minutes in a buffer solution with basic pH (7.8). Anti-BDNF and anti-NGF antibodies (Invitrogen, Rabbit Polyclonal Antibody) were incubated

for 48 minutes at 37°C with a dilution 1:100. For signal detection procedure, OptiView DAB IHC detection kit was used. At the end counterstain was performed with Hematoxilin for 8 minutes.
The examination under the microscope was carried out using an ordinary scale ranging from 0 (completely negative) to 3 (strong expression). NGF and BDNF show cytoplasmatic dot-like expression. In the analysis this characteristic was taken into consideration and the evaluating score was created considering both signal intensity and number of cells from the lesion that expressed the neurotrophin.

Statistical analysis

Patients were grouped according to the HIV status expression and clinical parameters were compared between the two groups. The Shapiro–Wilk test was performed to test for normality. When normally distributed, continuous variables were compared by Student's t-test, otherwise by Kruskal–Wallis test; categorical variables were compared by Pearson chi-square test. Furthermore, differences in the expression of NGF and BDNF were evaluated between the two groups. A p value <0.05 was considered as significant. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) Statistics v. 19 (IBM Inc., Armonk, NY, USA).

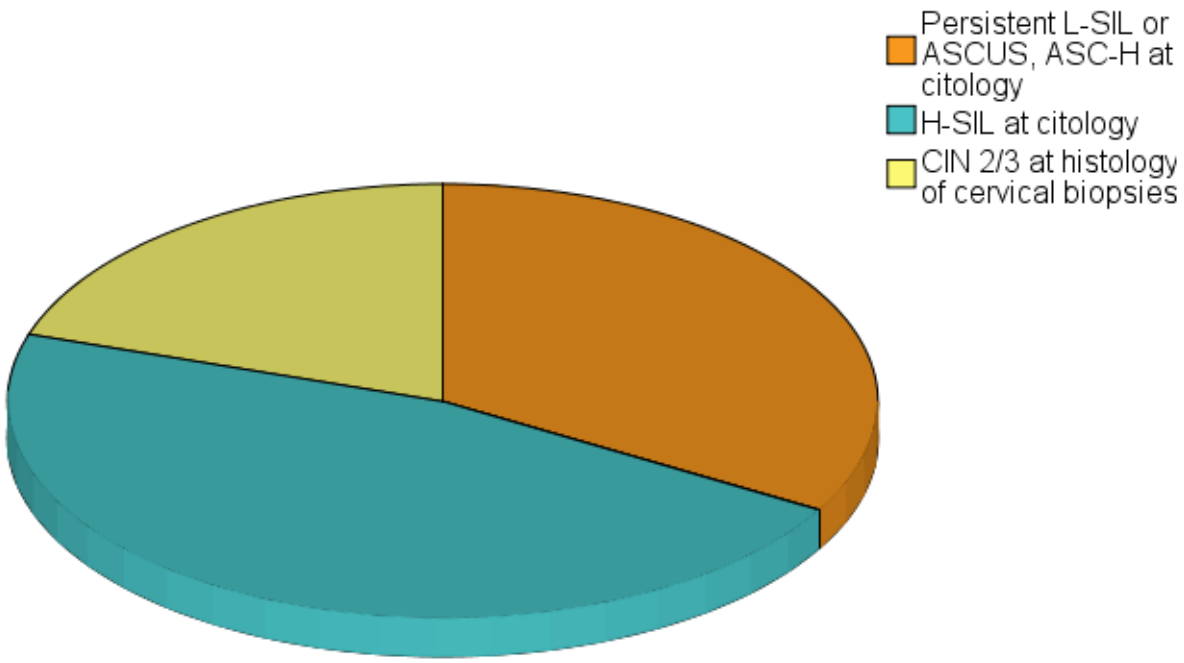
CHAPTER IV

RESULTS

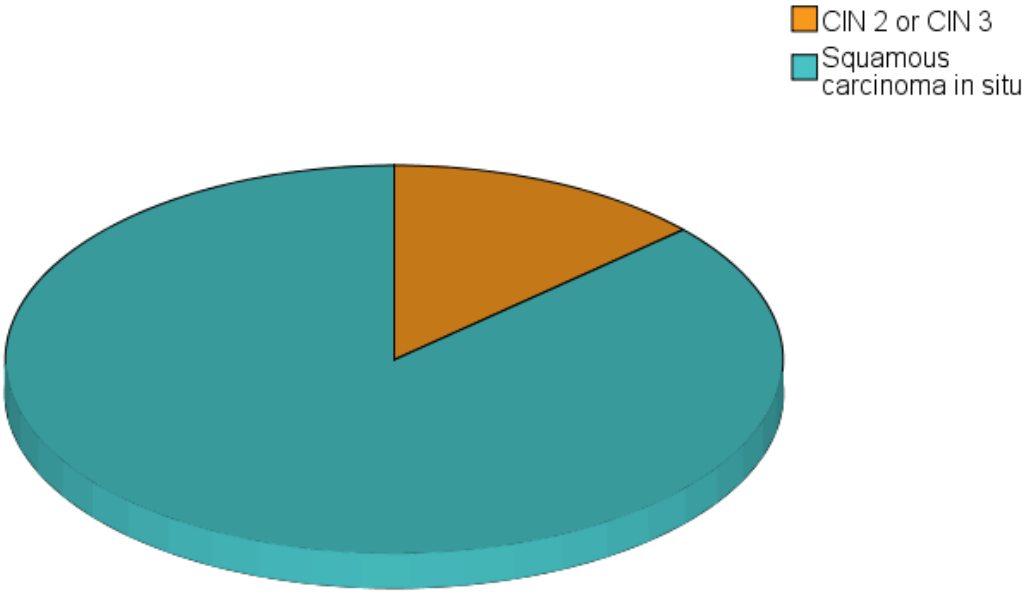
We included in our analysis 30 women matching the inclusion criteria: 15 HIV infected patients who were matched with 15 HIV negative patients. HIV patients and control group did not differ for mean age, parity, BMI, smoking status, and HPV-HR status. 86.7% of HIV positive women were on ART at time of inclusion in the study, whereas no HIV negative patient was on ART. The most common indication for LEEP was H-SIL at citology for both HIV positive (66.7%) and negative (46.7%) women. The most common histopathological finding in LEEP specimens was cervical squamous carcinoma in situ in both groups (86.7% in HIV positive women and 80% in the control group).

Characteristics	HIV + (n=15)	HIV – (n=15)	p
Age (years)	35.13 (5.08)	36.93 (6.54)	0.407
Parity	0.93 (0.96)	0.80 (0.68)	0.664
Smoke	6 (40%)	7 (46.7%)	χ^2=0.137; p=0.713
BMI (kg/m^2)	28.58 (3.58)	28.80 (3.37)	0.868
ART	13 (86.7%)	0	
HPV-HR	10 (66.7%)	13 (86.7%)	χ^2=1.677; p=0.195
Indication for LEEP*	1. 4 (26.7%) 2. 10 (66.7%) 3. 1 (6.7%)	1. 5 (33.3%) 2. 7 (46.7%) 3. 3 (20%)	
LEEP histological evaluation°	1. 2 (13.3%) 2. 13 (86.7%)	1. 3 (20%) 2. 12 (80%)	

Data given as mean; standard deviation or n (%). HIV: Human Immunodeficiency Virus; BMI: Body Mass Index; ART: antiretroviral therapy; HPV-HR: human papillomavirus high-risk genotype; LEEP: Loop Electrosurgical Excision Procedure. * 1. Persistent L-SIL or ASCUS, ASC-H at citology; 2. H-SIL at citology; 3. CIN 2/3 at histology of cervical biopsies during colposcopy exam. ° 1.CIN 2/3; 2. Squamous carcinoma in situ

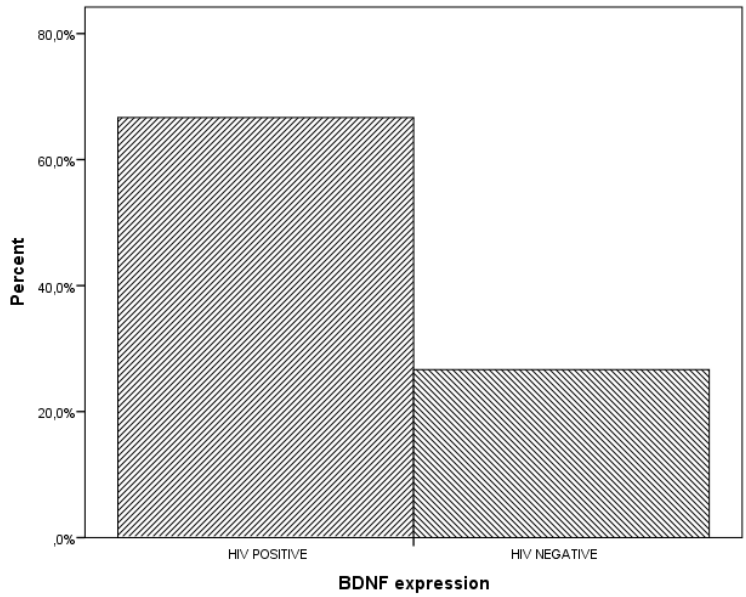


Indication for Loop Electrosurgical Excision Procedure in cases of suspected cervical preinvasive or invasive lesions in HIV positive women

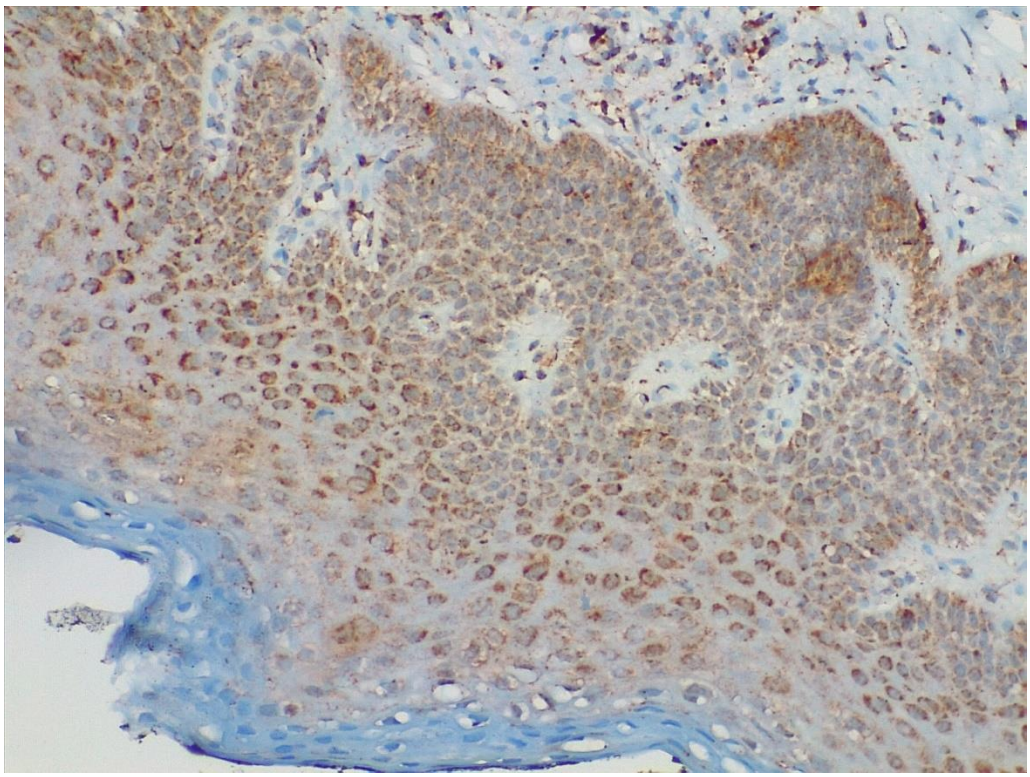


Histological findings in specimens from LEEPs in HIV positive women with suspected cervical invasive lesions

Immunochemistry analysis showed that BDNF expression was significantly higher in cervical preinvasive and invasive lesions from HIV positive women than in cervical lesions from HIV negative women. In particular, BDNF was expressed in 10/15 (66.7%) HIV positive patients and in 4/15 (26.7%) HIV negative patients (χ^2=4.821; p=0.028).

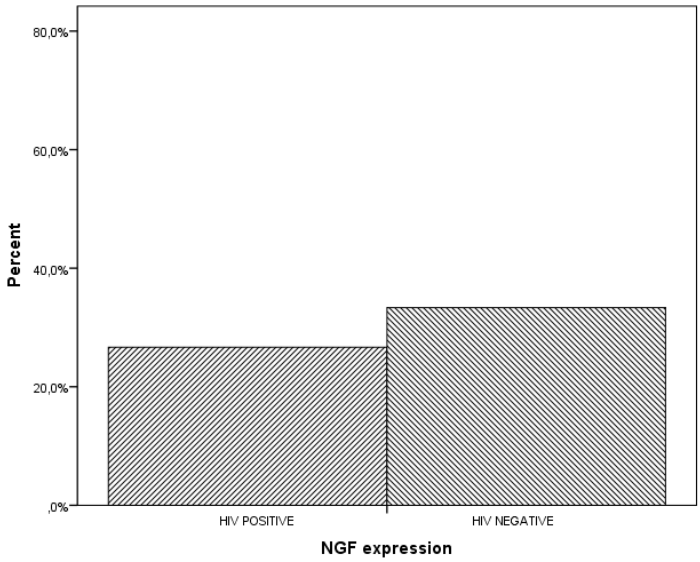


BDNF expression in specimens from pre-invasive and invasive cervical cancer according to the HIV status

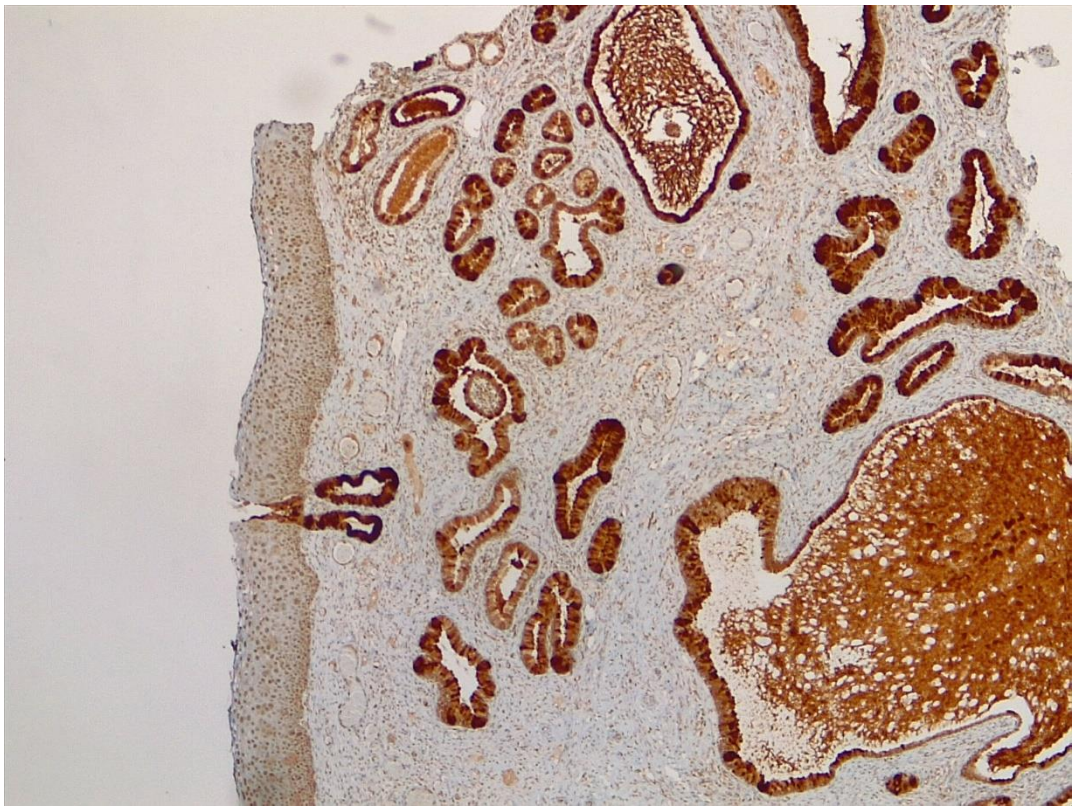


Immunohistochemistry: BDNF positive expression in cervical cancer

Immunochemistry analysis showed that NGF expression was not significantly higher in cervical preinvasive and invasive lesions from HIV positive women than in cervical lesions from HIV negative women. In particular, NGF was expressed in 4/15 (26.7%) HIV positive patients and in 5/15 (33.3%) HIV negative patients (χ^2=0.159; p=0.690).



NGF expression in specimens from pre-invasive and invasive cervical cancer according to the HIV status

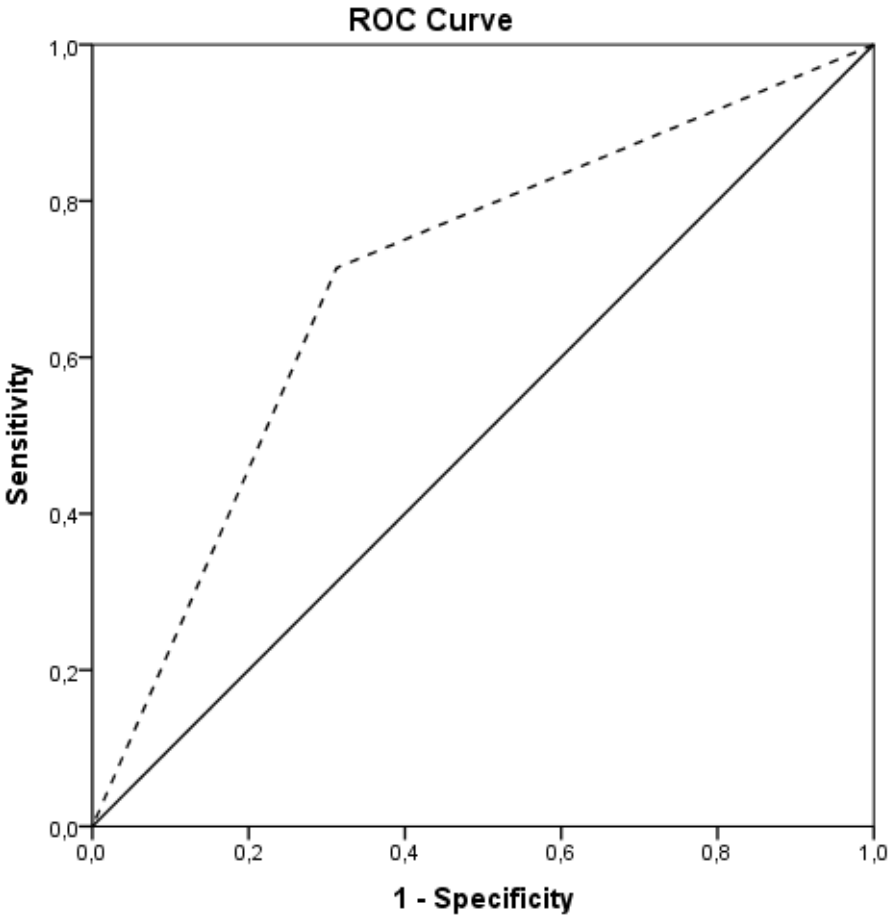


Immunohistochemistry: NGF positive expression in cervical cancer

We tested the role of HIV status in the expression of neurotrophins in pre-invasive and invasive cervical cancer. Therefore, we performed a logistic regression analysis, including the following variables: HIV status, presence of HPV-HR, smoking status, BMI, age, histological diagnosis at LEEP (1. CIN 2/3; 2. squamous carcinoma in situ).

Logistic regression analysis showed that HIV status is an independent predictor of BDNF expression in pre-invasive and invasive cervical cancer when considered alone (crude OR 5.5, 95 % CI 1.145-18.412; p= 0.033) and when anlyzed with other co-factors (adjusted OR 6.356, 95 CI 1.103-20.487; p= 0.046).

Finally, receiver operating characteristics (ROC) analysis demonstrated that HIV status is a good prediction for BDNF expression even if this results did not reach statistical significance (AUC 0.701; 95 CI 0.508-0.893; p=0.061).



ROC curve analysis: the prediction of BDNF expression according to the HIV status

CHAPTER V

DISCUSSION

Our study demonstrated that BDNF expression in pre-invasive and invasive cervical cancer is higher in HIV infected women than in non-infected controls. We showed that this co-relation is independent from patient characteristics, type of displastic lesion (CIN 2/3 vs ca in situ) and presence of HPV-HR genotype. On the other hand, NGF expression did not show any difference according to the HIV status.

Authors have reported the role of neurotrophins, in particular BDNF, in the association between HIV infection and neurocognitive disorders among infected patients. Indeed, neurons exposed to gp120 exhibit lower concentrations of mBDNF and higher levels of proBDNF than controls.

BDNF plays a critical role in learning and memory. Increased BDNF levels have been shown to decrease the odds of developing HIV-associated neurocognitive disorder (HAND). Abassi et al in a Ugandan population, showed lower CSF BDNF levels in HIV patients with dementia compared with HIV-positive individuals without dementia[140]. Falasca et al were able to correlate BDNF levels with different domains of neurocognition[141]. They showed a significant correlation between reduced serum BDNF levels and poor performance on the Grooved Pegboard test for the dominant hand test but no association between BDNF serum levels and attention, executive function and working memory availability.

HIV impacts negatively on dopaminergic (DA) neurons within the fronto-striato-thalamic system. This causes specific cognitive, motor and behavioral deficits in HIV infected patients[142]. A decrease in dopamine in the substantia nigra has been associated with poorer cognitive function across specific domains like speed of processing, memory, learning and verbal fluency[143]. In gp120-treated rats, DA levels were found to be lower than controls in the dorsal striatum and there was loss of nigrostriatal fibers due to intra-striatal gp120[144]. Recent studies seem to be contradictory showing an increase in DA levels in the CSF of therapy-naive HIV patients. This increase in DA levels was in the asymptomatic stage of infection and could later exert toxic effects in DA neurons due to the oxidative properties of catecholamines[145]. Increased cytotoxicity of the DA neurons eventually leads to dopamine deficits that may exacerbate the progression of HAND[146].

In peripheral nervous system, HIV infection has been associated with the overexpression of neurotrophins. Authors suggested that neurotrophins, in particular BDNF, can have a key role in the development of HIV-associated neurotoxicity. While BDNF in central nervous system have a neurotrophic role, preventing neurons and microglia cells from apoptosis, is has been showed that BDNF has a role in the inflammation in the peripheral tissues, uperegulating chemokines.

We reported studies demonstrating that HIV infection causes the upregulation of BDNF expression via gp120 and Tat proteins. Indeed, gp120-upregulated BDNF expression has been shown to contribute to the development of HIV-associated pain through wnt/β-catenin signaling in murine derived microglial cell lines[146].

Furthermore, recent studies have shown that both NGF and BDNF have an indirect angiogenic role, mediated by VEGF modulation in different cellular models. Another key point is that plasmatic levels of VEGF are lower in deficient BDNF animals compared to wild type animals. All these antecedents indicate that NTs not only act directly in vascular cells, but also affect several cell types by increasing VEGF expression and therefore their angiogenesis potential.

Receptor tyrosine kinase B (TrkB) is a transmembrane protein that is a specific receptor for brain-derived neurotrophic factor (BDNF) and belongs to the neurotrophic factor receptor Trk family, which consists of extracellular glycosylated polypeptides, transmembrane region and cytoplasmic tyrosine kinase domain. TrkB can be activated by BNDF or neurotrophic factor (NT) −4/5.

Activated TrKB plays an important role in the development and maturation of the nervous system. However, there is mounting evidence that TrKB plays an important role in promoting tumor formation and metastasis in some malignancies. Previous findings showed that the upregulation of the BDNF/TrKB pathway promotes the epithelial-mesenchymal transition, as well as the migration and invasion of cervical cancer. BDNF/TRKB increases cell proliferation apparently involving ERK and AKT signaling pathways. Furthermore, the activated PI3K/AKT pathway by TrkB/BDNF can block the activation of caspase-3, thereby inducing tolerance to apoptosis. In addition, normal epithelial cells did not express or lowly expressed BDNF and TrkB, while the expression of BDNF and TrkB in various cancer cells was significantly enhanced. However, whether the TrkB/BDNF signaling pathway activates, as well as how to activate the downstream pathway and mediate cervical cancer cell anoikis tolerance remains to be clarified. Therefore, examinig the TrkB/BDNF pathway can be useful in determining the molecular mechanism of cervical cancer metastasis to develop the corresponding drugs and improve the survival rate of patients.

This is the first study to evaluate the immunohistochemistry expression of BDNF in pre-invasive and invasive cervical cancer according to the HIV infection, possibly highlighting the role of BDNF as mediator of cervical dysplastic and neoplastic progression, enhanced by HIV infection. In our proposed model, HIV infection causes an overexpression of BDNF in cervical tissues. Increased BDNF expression can be responsible for accelerated progression of HPV-related dysplastic lesions, explaining the reason for the increased incidence of cervical cancer cases, the reduced interval between HPV infection and the onset of invasive cervical cancer and the increased incidence of recurrences among HIV positive women.

The main limitation of the present study is the limited number of included case and the missing information regarding follow-ups of included patients. Indeed, at the moment, the investigation is still ongoing and more robust data are expected from a larger cohort.

These results are interesting, since the recent interest in therapeutic strategies for cancers involving TRK inhibitors in malignancies with TRK fusions. TRK fusions (fusions between one of the NTRK genes and a 5′ partner gene) arise from intrachromosomal or interchromosomal rearrangements that collectively lead to the expression of a chimeric oncoprotein characterised by ligand-independent kinase activation, which consequently drives oncogenesis[147]. TRK fusions are found in various adult and paediatric cancers. These cancers include rare tumours (eg, mammary analogue secretory carcinoma, secretory breast carcinoma,4 infantile fibrosarcoma, and cellular congenital mesoblastic nephroma) in which TRK fusions are found in the majority of cases, and more common cancers (eg, lung, breast, and gastrointestinal carcinomas, melanomas, and sarcomas) in which TRK fusions are found at lower frequencies[148]. TRK fusions can be identified by DNA-based or RNA-based next-generation sequencing, or other assays such as fluorescence in-situ hybridisation (FISH) or immunohistochemistry[149]. Larotrectinib is a small molecule that binds to NTs receptors, thereby preventing neurotrophin-TRK interaction and TRK activation, which results in the induction of cellular apoptosis and the inhibition of cell growth[150,151]. Larotrectinib has become the first tyrosine kinase inhibitor to be granted approval by the US Food and Drug Administration (FDA) and the European Medicines Agency for a tumour-agnostic indication; specifically, the treatment of adult and paediatric patients with advanced solid tumours that harbour an NTRK gene fusion. In September 2021, the Italian Medicines Agency has approved Larotrectinib for the treatment of agnostic tumors with NTRK gene fusions.

Given that angiogenesis is a key feature in gynecological neoplasms, and NTs acts as direct and indirect angiogenic factors, it may be relevant to study whether TRK inhibitors could improve the efficacy of antiangiogenic drugs, especially in cervical cancers with a co-existing HIV infection.

Presently, no data are available on the efficacy of NTRK inhibitors in HIV infected patients with cancer, especially those diagnosed with cervical cancer.

To conclude, BDNF is overexpressed in pre-invasive and invasive cervical cancer in HIV positive women compared to HIV negative controls. Our results show that BDNF could play a key role as link between the pathways by which HIV and HPV interacts to accelerate cervical cancer progression and invasion. This data could be useful to better understand the role of neurotrophins in the etiopathology of cervical cancer and the possible therapeutic strategies to improve patients outcome.

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