The epidemiology of human papillomavirus and interaction with human immunodeficiency virus in Kenya.

Doctoral Thesis submitted to the Faculty of Medicine and Health Sciences, Ghent University

Dr. Hugo De Vuyst

Promotor: Prof. Dr. Marleen Temmerman
Dept of Obstetrics and Gynaecology, Ghent University

Co-promotor: Prof. Dr. John-Paul Bogers
Dept of Pathology, Antwerp University
The epidemiology of human papillomavirus and interaction with human immunodeficiency virus in Kenya. Doctoral Thesis submitted to the Faculty of Medicine and Health Sciences, Ghent University
Dr. Hugo De Vuyst

Promotor: Prof. Dr. Marleen Temmerman
Dept of Obstetrics and Gynaecology, Ghent University Hospital
Co-promotor: Prof. Dr. John-Paul Bogers
Dept of Pathology, Antwerp University

Deze publicatie is verschenen binnen de reeks “ICRH Monografieën”/ This title has been published in the series “ICRH Monographs”
ISBN 978 90 382 1188 6

International Centre for Reproductive Health Ghent (ICRH)
Ghent University (UGent)
De Pintelaan 185 P3
B- 9000 Ghent (Belgium)
www.icrh.org
TABLE OF CONTENTS

ABBREVIATIONS ................................................................................................... 7

1. INTRODUCTION ............................................................................................ 11
   1.1. The epidemiology of cervical cancer ....................................................... 11
   1.2. The human papillomavirus ....................................................................... 15
       1.2.1. Virological and biological aspects .................................................... 15
       1.2.2. Classification of HPV ....................................................................... 16
       1.2.3. Natural history of HPV infection and the causation of cervical cancer ................................................................................................ 19
       1.2.4. Immune response against HPV ......................................................... 24
       1.2.5. Detection of cervical HPV infection ................................................. 25
       1.2.6. Epidemiology of HPV ...................................................................... 29
   1.3. Prevention of cervical cancer ................................................................... 39
       1.3.1. Screening of cervical pre-cancer and cancer .................................... 39
       1.3.2. Vaccination against HPV ................................................................. 46
   1.4. The immunodeficiency virus epidemic in Africa ..................................... 51
       1.4.1. The HIV epidemic in sub-Saharan Africa ........................................ 51
       1.4.2. The HIV epidemic in Kenya ............................................................. 55
       1.4.3. Clinical and immunological classification of HIV / AIDS ............... 56
       1.4.4. Antiretroviral treatment .................................................................... 59
   1.5. HIV, HPV and cervical dysplasia and cancer in the era of HAART ...... 63

2. OBJECTIVES AND METHODOLOGY ........................................................... 93
   2.1. General Objective ..................................................................................... 93
   2.2. Specific Objectives ................................................................................... 93
   2.3. Study sites and study development .......................................................... 94
   2.4. Dissemination of results ........................................................................... 97
       2.4.1. Presentations at Scientific conferences ............................................. 97
       2.4.2. Publications in Peer reviewed journals ............................................. 99

3. RESULTS ..................................................................................................... 101
   3.1. Distribution of Human Papillomavirus in a Family Planning Population in Nairobi, Kenya. .......................................................... 101
   3.2. Human papillomavirus infection in Mombasa, Kenya: a population-based study among family planning attenders .................... 109
   3.3. HIV and cervical cancer in Kenya. ............................................................. 119
3.4. Impact of HIV infection on invasive cervical cancer in Kenyan women. ................................................................. 131

3.5. Human papillomavirus types in women with invasive cervical carcinoma by HIV status in Kenya. ....................................................... 139

3.6. Comparison of pap smear, visual inspection with acetic acid, human papillomavirus DNA-PCR testing and cervicography............. 149

4. DISCUSSION................................................................................................ 159

4.1. Contribution of this work to the field..................................................... 159

4.2. HPV in Kenya .................................................................................... 160

4.3. Prevention of cervical cancer in Kenya................................................. 168
   4.3.1. Prevention of cervical cancer through alternative screening methods: ................................................................. 168
   4.3.2. Prevention of cervical cancer through HPV vaccination: ........ 170

4.4. Conclusions ............................................................................................ 171

4.5. Recommendations .................................................................................. 173

5. EXECUTIVE SUMMARY .............................................................................. 175

5.1. Context and Objectives .......................................................................... 175

5.2. Results .................................................................................................... 176
   5.2.1. HPV in Kenya .............................................................................. 176
   5.2.2. Prevention of cervical cancer in Kenya through HPV vaccination .............................................................................. 178
   5.2.3. Prevention of cervical cancer in Kenya through alternative cervical screening methodologies ................................................... 179

5.3. Conclusions and recommendations ........................................................ 179

6. SAMENVATTING .......................................................................................... 181

6.1. Context en Objectieven ........................................................................... 181

6.2. Resultaten ................................................................................................ 182
   6.2.1. HPV in Kenia .............................................................................. 182
   6.2.2. Preventie van cervixkanker in Kenia door middel van HPV vaccinatie: ................................................................. 185
   6.2.3. Preventie van cervixkanker in Kenia door middel van alternatieve manieren van screening............................................... 185

6.3. Besluiten en aanbevelingen ....................................................................... 186

7. RÉSUMÉ ...................................................................................................... 189
7.1. Contexhe et Objectifs ................................................................. 189
7.2. Résultats ................................................................................. 190
  7.2.1. HPV au Kenya ................................................................. 190
  7.2.2. Prévention du cancer du col de l’utérus au Kenya grâce à la
         vaccination: ................................................................. 193
  7.2.3. Prévention du cancer du col au Kenya grâce à des méthodes
         de dépistage alternatives .............................................. 193
7.3. Conclusions et recommendations .............................................. 194

8. ACKNOWLEDGEMENTS ............................................................. 197

9. REFERENCE LIST ................................................................. 199

10. ANNEXES .................................................................................. 219
  10.1. ANNEX Curriculum Vitae .................................................... 219
  10.2. ANNEX Supporting Paper .................................................. 227
  10.3. ANNEX Supporting Paper .................................................. 235
  10.4. ANNEX Supporting paper .................................................. 253
  10.5. ANNEX VIA Atlas ............................................................... 261
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>3TC</td>
<td>Lamivudine</td>
</tr>
<tr>
<td>AAT</td>
<td>Acetic acid test</td>
</tr>
<tr>
<td>ACCP</td>
<td>Alliance for Cervical Cancer Prevention</td>
</tr>
<tr>
<td>ADC</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>ART</td>
<td>Anti-retroviral treatment</td>
</tr>
<tr>
<td>ARV</td>
<td>Anti-retroviral</td>
</tr>
<tr>
<td>ASCUS</td>
<td>Atypical squamous cells of undetermined significance</td>
</tr>
<tr>
<td>ASIR</td>
<td>Age standardised incidence rate</td>
</tr>
<tr>
<td>AZT</td>
<td>Zidovudine</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>CHW</td>
<td>Community health workers</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CIN(1-2-3)</td>
<td>Cervical intra-epithelial neoplasia grade 1-2-3</td>
</tr>
<tr>
<td>CIS</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>CPGH</td>
<td>Coast Provincial General Hospital</td>
</tr>
<tr>
<td>d4T</td>
<td>Stavudine</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DVI</td>
<td>Direct visual inspection</td>
</tr>
<tr>
<td>EFV</td>
<td>Efavirenz</td>
</tr>
<tr>
<td>E-proteins</td>
<td>Early proteins (HPV)</td>
</tr>
<tr>
<td>FDC</td>
<td>Fixed-dose combination</td>
</tr>
<tr>
<td>FP</td>
<td>Family planning</td>
</tr>
<tr>
<td>FPAK</td>
<td>The Family Planning Association of Kenya</td>
</tr>
<tr>
<td>FTC</td>
<td>Emtricitabine</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly active antiretroviral treatment</td>
</tr>
<tr>
<td>HC 1-2</td>
<td>Hybrid Capture (1–2) HPV test</td>
</tr>
<tr>
<td>HERS</td>
<td>HIV epidemiology research study</td>
</tr>
<tr>
<td>HPV</td>
<td>Human papillomavirus</td>
</tr>
<tr>
<td>HPV-X</td>
<td>Uncharacterized HPV type</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HR-HPV</td>
<td>High-risk HPV</td>
</tr>
<tr>
<td>HSIL</td>
<td>High grade squamous intra-epithelial lesion</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>ICC</td>
<td>Invasive cervical carcinoma</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IHPS</td>
<td>IARC HPV prevalence surveys</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital (Nairobi)</td>
</tr>
<tr>
<td>LBA</td>
<td>Line Blot Assay</td>
</tr>
<tr>
<td>LiPA</td>
<td>Line Probe Assay</td>
</tr>
<tr>
<td>LBC</td>
<td>Liquid-based cytology</td>
</tr>
<tr>
<td>L-proteins</td>
<td>Late proteins (HPV)</td>
</tr>
<tr>
<td>LR-HPV</td>
<td>Low-risk HPV</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low grade squamous intra-epithelial lesions</td>
</tr>
<tr>
<td>MT</td>
<td>Multiple-type (HPV infections)</td>
</tr>
<tr>
<td>NNRTI</td>
<td>non-nucleoside reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>NRTI</td>
<td>Nucleoside reverse transcriptase inhibitors</td>
</tr>
<tr>
<td>NVP</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>PATH</td>
<td>Programme for Appropriate technology in Health</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PHIV</td>
<td>People with HIV</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>SCJ</td>
<td>Squamocolumnar junction</td>
</tr>
<tr>
<td>START</td>
<td>Screening Technologies to Advance Rapid Testing</td>
</tr>
<tr>
<td>STD</td>
<td>Sexually transmitted disease</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenofovir</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lymphocyte count</td>
</tr>
<tr>
<td>Th1</td>
<td>Cell-mediated immunity</td>
</tr>
<tr>
<td>Th2</td>
<td>Anti-body mediated (humoral) immunity</td>
</tr>
<tr>
<td>VIA</td>
<td>Visual inspection with acetic acid</td>
</tr>
<tr>
<td>VILI</td>
<td>Visual inspection with Lugol’s iodine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>VLIR</td>
<td>Flemish Interuniversity Council</td>
</tr>
<tr>
<td>VLP</td>
<td>Virus-like particles</td>
</tr>
<tr>
<td>WHIV</td>
<td>Women with HIV infection</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WISH</td>
<td>Women’s interagency HIV study</td>
</tr>
</tbody>
</table>
1. INTRODUCTION

References from the “Introduction” section are listed in Section 9., except for chapter 1.5. “HIV, HPV and cervical dysplasia and cancer in the era of HAART”, for which the references are listed at the end of the same chapter.

1.1. The epidemiology of cervical cancer

Global burden

Worldwide, it is estimated that over 493,000 new cases (comprising both SCC and adenocarcinoma) and 273,000 deaths annually are due to cervical cancer (Table 1.1.) [Ferlay et al., 2005]. Cancer of the cervix is the second most common cause of death from cancer in women globally. The majority of the cancer burden occurs among women living in developing countries where approximately 83% of new cases, deaths occur each year and cervical cancer surpasses breast-cancer as the leading cause of cancer deaths in women [Ferlay et al., 2005]. A majority of these cases occur in Asia (approximately 55%) due primarily to the large burden of cervical cancer in India, which alone accounts for approximately 27% of the global figures. Africa accounts for 10% of all incident cases.

Table 1.1. The incidence of cervical cancer by region (Source: GLOBOCAN 2002 data [Ferlay 2004])

<table>
<thead>
<tr>
<th>Region</th>
<th>New Cases Annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global</td>
<td>493,967</td>
</tr>
<tr>
<td>Africa</td>
<td>78,791</td>
</tr>
<tr>
<td>Central, South America &amp; Caribbean</td>
<td>71,862</td>
</tr>
<tr>
<td>North America</td>
<td>14,670</td>
</tr>
<tr>
<td>Asia</td>
<td>265,714</td>
</tr>
<tr>
<td>Region</td>
<td>Incidence</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Europe</td>
<td>59,929</td>
</tr>
<tr>
<td>Australia &amp; New Zealand</td>
<td>1,063</td>
</tr>
<tr>
<td>Melanesia, Micronesia &amp; Polynesia</td>
<td>941</td>
</tr>
</tbody>
</table>

Figure 1.1. Worldwide age-standardized cervical cancer incidence and mortality rates for cervical cancer per 100,000 (Source: GLOBOCAN 2002 data [Ferlay 2004])

Figure 1.1. shows worldwide age-standardized cervical cancer incidence and mortality rates. It can be remarked that the incidence rates are higher for less developed countries in general, compared to more developed countries.

In general there is a correlation between incidence and mortality across all regions, but a disproportionately higher mortality rate is evident in Africa. A high degree of regional variation in incidence and mortality rates is also apparent.

Cervical cancer is generally a disease of middle and older age, peaking among women aged 50 - 70 years. The same pattern of increasing incidence with age is observed in all regions, despite underlying differences in incidence rates [Ferlay et al., 2005].
The lifetime (or cumulative) risk of developing cervical cancer mirrors the age-standardized incidence rates (ASIR) and shows a high global variation (Figure 1.2). Risk estimates correlate broadly with the availability of organized screening programs. The lowest lifetime risk were again seen in more developed countries [Ferlay et al., 2005].

Figure 1.2. Cumulative risk of cervical cancer by region. (Source: GLOBOCAN 2002 data [Ferlay et al., 2005])

Prevalence, incidence and mortality rates in sub-Saharan Africa:
A full understanding of the burden of cervical cancer in Africa is limited by scarce national registry data and estimation of the burden of disease through frequency data and averages from neighboring countries (Figure 1.4). However, it is clear that high level of exposure to HPV, absence of screening programs and poor access to appropriate treatment have resulted in the highest cervical cancer rates in the world among sub-Saharan African women (Figure 1.3). East Africa has notably high rates of cervical cancer incidence (ASIR 42.7), as well as mortality rates (ASR 34.6) (Figure 1.1), and also has the highest cumulative risk (3.2) of developing cervical cancer (0-65 years) in the world, with cumulative risk reaching 5.2 in Tanzania. A limited population based registry, covering a rural population of 623,000 reported a ASIR of
25.9 for cervical cancer for the period 1998 – 2000 [Parkin et al., 2003]. The cumulative risk is estimated at 2.2 for Kenyan women [Ferlay et al., 2005].

Figure 1.3: Incidence of cervical cancer in the African region

Figure 1.4: Quality of country-specific cancer incidence data for Africa (GLOBOCAN, [Ferlay et al., 2005]):
1.2. The human papillomavirus

Figure 1.5.: Human papillomavirus:

![Human papillomavirus image](www.hopkins.org) (HPV up close. Image courtesy of Laboratory of Tumor Virus Biology, NIH's National Cancer Institute.

1.2.1. Virological and biological aspects

Papillomaviruses are small, double stranded, non-enveloped circular DNA viruses, members of the papovaviridae family, which can cause a variety of proliferative epithelial lesions in mammals, referred to as papillomas or warts. HPV infects basal cells from mucosal epithelia through micro-abrasions, caused by micro-trauma. HPV starts its life cycle in episomal form with limited expression of E “early” viral genes. E1 and E2 proteins mediate HPV DNA replication through recruitment of cell polymerases and accessory proteins [Frattini and Laimins, 1994;Mohr et al., 1990]. Subsequently, genes are expressed that have a proliferation stimulating activity. E5 stimulates cell growth and prevents apoptosis following DNA damage [Zhang et al., 2002]. E6 and E7 are potent viral oncoproteins that immortalize human cell types, through suppression or inactivation of tumor suppressor proteins p53 (E6) and pRb (E7). At this stage, the basal cells are blocked from exit from the
cell cycle, experience an enhanced proliferation and contain hundreds of copies of HPV genomes per cell [Kanodia et al., 2007]. Some of the daughter cells move to the suprabasal layers, where they start differentiating, however in continued active cell cycle due to the E7 protein. The cell continues to replicate HPV genomes and starts to express L “late” viral genes, probably mediated by E4, E5. L1 and L2 are viral capsid proteins that self-assemble into icosahedral capsids containing the viral genome. L1 is the main capsid and also largest and containing the most immunogenic epitopes. Mature viruses are finally released from the uppermost layers of the epithelium at the end of the keratinocyte lifespan. Several cycles of reinfection are possible. As a result of viral oncogene activity, HPV interacts with numerous cellular targets to disrupt cell cycle control, resulting in increased proliferation, decreased apoptosis and progressive accumulation of cellular DNA damage [Unger and Barr, 2004]. In neoplasia or cancer, HPV DNA is mostly integrated in the host cell genome [zur Hausen, 1996]. This transformation is accompanied by suppression of E2 and subsequent enhanced expression of E6 and E7, which keeps the cell immortalised and causes an accumulation of genetic changes.

1.2.2. Classification of HPV

Taxonomy and phylogeny

Papillomavirus classification is based on the species of origin (human, bovine, rabbit, etc.) and, within a species, by genetic comparison of the E6, E7 and L1 genes [Van Ranst et al., 1992]. Several hundred of types of the virus have been partially identified by short DNA fragments [de Villiers et al., 2004]. Each HPV type either infects cutaneous or mucosal epithelium depending upon its cell specificity. The HPV types which infect the genital mucosa can be further categorized epidemiologically as HR-HPV or low-risk (LR) HPV [Munoz et al., 2003]. The viral L1 gene is well conserved and forms the taxonomic basis for type identification using DNA sequencing technology [de Villiers et al., 2004]. Of the 15 high-risk (HR) types identified (see Table 2.1.), 11 belong to two important phylogenetically related families: those types related to HPV-16 (types 31, 33, 35, 52,
HPV is a stable virus and more than 100 distinct genotypes of HPV have been completely described and identified from nucleic acid variation in specific regions of the viral DNA [de Villiers et al., 2004]. By definition, DNA sequences in the L1 region of each genotype differ by at least 10%.

Each genotype comprises a number of subtypes showing between 2% and 10% of nucleotide sequence difference. Within subtypes, variants exist, with a maximum sequence divergence of 2% [de Villiers et al., 2004]. Most of the available evidence related to variants focuses on HPV-16 and limited information is available on HR-HPV types 18, 31, 35, and 52. The association between infection with a specific subtype or variant of HPV (mainly HPV-16) and the risk of cervical neoplasia remains unclear to date.

**Epidemiological classification of HPV types**

HPV types are classified in terms of their oncogenic potential. Approximately 40 HPV types infect the genital tract. The HR-HPV types are strongly associated with
cervical cancer, notably HPV-16 and 18, whereas the low risk (LR)-HPV types are not associated with cervical cancer and generally cause benign conditions, e.g., genital warts (HPV-6 and 11).

The primary evidence for the causal relationship between HPV and cervical cancer came from a large worldwide series of case-control studies conducted by the International Agency for Research on Cancer (IARC), published between 1992 and 2004, that allow calculation of type-specific odds ratios [Munoz et al., 2003; Munoz et al., 2004]. Table 1.2. shows the epidemiological classification of HPV types. There is a large degree of concordance between phylogenetic and epidemiologic studies that confirm the risk category attributed to individual HPV types [de Villiers et al., 2004; Munoz et al., 2003; Schiffman and Castle, 2003].

Table 1.2: Epidemiological classification of HPV types (Source: Munoz 2006)

<table>
<thead>
<tr>
<th>Group</th>
<th>HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Established high-risk</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59</td>
</tr>
<tr>
<td>Probable high-risk</td>
<td>26, 53, 66, 68, 73 and 82</td>
</tr>
<tr>
<td>Established low-risk</td>
<td>6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108</td>
</tr>
</tbody>
</table>
1.2.3. Natural history of HPV infection and the causation of cervical cancer

As mentioned earlier, HR-HPV infection is necessary for the development of the majority of cases of cervical cancer and its immediate precursor CIN3 [Munoz et al., 2003; Walboomers et al., 1999]. Although most women will at some time have been infected with HPV, few will progress to invasive disease. Therefore, it is crucial to have a good understanding of the natural history of HPV infection, and its relationship to the development of epithelial abnormalities.
Squamocolumnar junction, Metaplasia and the Transformation Zone

Figure 1.8: Position of the squamocolumnar junction and the transformation zone

Used with courtesy from the Program for Appropriate Technology in Health (PATH). Seattle, WA: PATH

The squamocolumnar junction is the line where ectocervical squamous epithelium meets the endocervical columnar epithelium. The location of the squamocolumnar junction in relation to the external os varies, mainly depending upon factors such as age and hormonal status. During childhood and perimenarche, it is located at the external os. After puberty and during the reproductive period, the female genital organs grow under the influence of estrogen. Thus, the cervix enlarges and the endocervical canal elongates. This leads to the eversion of the columnar epithelium onto the ectocervix, particularly on the anterior and posterior lips, resulting in ectropion or ectopy.

The buffer action of the mucus covering the columnar cells is interfered with when the everted columnar epithelium is exposed to the acidic vaginal environment. This leads to the destruction and eventual replacement of the columnar epithelium by a newly formed metaplastic squamous epithelium. As a woman passes through her reproductive life to the perimenopausal age, the location of the squamocolumnar junction progressively starts moving on the ectocervix towards the external os. In postmenopausal women, the squamocolumnar junction is located in the endocervical canal.
Metaplasia of the endocervix results in substitution of the one layer thick glandular layering by a stratified squamous epithelium. In a very small minority of women, the squamous metaplasia may turn into a dysplastic epithelium (an altered epithelium showing precancerous cellular changes), due to infection with HR-HPV types.

The transformation zone is the area of the cervix where the columnar epithelium has been replaced and/or is being replaced by the metaplastic squamous epithelium. With the naked eye, one can identify the inner border of the transformation zone by tracing the squamocolumnar junction and the outer border by locating the distal most crypt openings. In premenopausal women, the transformation zone is primarily located on the ectocervix. After menopause, and through old age, the cervix shrinks with the decreasing levels of oestrogens. Consequently, the transformation zone may move partially, and later fully, into the endocervical canal, thus hiding it for visual inspection. Almost all cervical neoplasia occurs in this transformation zone [Sankaranarayanan et al. 2003].

**Intra-epithelial microscopic abnormalities**

As explained earlier, expression of viral oncogenes E2, E5, E6 and E7 causes epithelial basal cells to become immortal and gradually occupy larger proportions of the epithelial layers. The spectrum of HPV-related epithelial abnormalities leading to cancer was formerly considered a step-wise progression of increasingly severe intraepithelial neoplasia—grade 1 to grade 2 to grade 3 to cancer. This is mostly a histopathological concept, while it becomes gradually more evident that HPV-related parameters might be more important than the presence or absence of mild microscopic evidence of infection in the prediction of risk of cancer [Moscicki et al., 2006]. The most important viral characteristics are genotype and length of persistence.

**Persistent HPV infection**

Persistent infection with a HR HPV type is necessary for the development of CIN2-3 and invasive disease. Persistence can be broadly defined as detection of the same HPV type two or more times with a given time interval between the examinations. There is no agreed cut-off point between transience and persistence. The observed median time to clearance of prevalent infections ranges from four to six months to up to two years in different studies, depending on follow-up strategies, age of
populations (longer in older women) and definitions (e.g., whether one or two negative tests are required to define clearance). Nonetheless, 70 to 80% of HPV infections are eventually cleared in one year’s time [Schiffman et al., 2005; Strickler et al., 2003]. Some data suggest that HPV-16 persists longer, on average, than any other type [Schiffman et al., 2005]. Any other inter-type differences are subtle, as far as it has been possible to assess, due to the lower prevalences of these types in the population [Schiffman et al., 2005][Cuschieri et al., 2005]. Within a cohort of 10,049 women from the population in Guanacaste, Costa Rica, followed up for 5 years, Schiffman et al. were able to demonstrate the unique potential of HPV-16 to persist (29% of prevalent infections), compared to other types within the same phylogenetic group (e.g. 16.5% and 14.9% for types 58 and 31, respectively), other HR groups (15.7% and 9.4% for types 18 and 45, respectively) or LR groups (10% and 17% for types 6 and 54, respectively).

However, it remains to be determined whether persistent infections are characterized by the continuing detection of HPV, or by a state of latency during which the virus remains undetectable only to reappear later [Wallin et al., 1999]. A mechanism for latency has not been established so far, nor is it clear whether the differences between a latent and active cervical infection are qualitative or quantitative. A clearer understanding of these issues is essential for the effective implementation of screening strategies, which include HPV testing.

**Progression of persistently infected epithelium to cervical pre-cancer**

Histologic diagnosis of CIN3 is now considered to be the true intraepithelial precursor to invasive cancer. It can be reliably distinguished from recently acquired HPV infection and it is a good indicator of subsequent cancer risk. In contrast, there is substantial heterogeneity in the microscopic diagnosis and biological meaning of CIN2 lesions: some represent acute HPV infections that will regress, while others are incipient pre-cancer that will persist and progress, while some LR-HPV types are also capable of producing lesions diagnosed as CIN2.

Using cross-sectional data, the modal time between HPV infection and CIN3 has been calculated to be 7–15 years with infection occurring in the later teens or early twenties and CIN3 diagnosis peaking around 25–30 years of age [Bosch and de Sanjose, 2003]. However, prospective cohorts with careful, intensive follow-up are
documenting rapid development of CIN2 and 3, sometimes within a few months after incident infection [Winer et al., 2005]. The biological meaning of these “early” CIN3 diagnoses is unknown. It is plausible that many of these would regress, however, observational studies of these lesions would be unethical [Moscicki et al., 2006].

The influence of HPV viral load
Among women who test positive for high-risk HPV types, cytological abnormality is more common in those with a high viral load [Woodman et al., 2007]. However, the relationship between viral load and disease seems rather complex. Many, but not all cross-sectional studies reported an increase in viral load with increasing disease severity [Heard et al., 2000;Lillo et al., 2005;Lorincz et al., 2002;Swan et al., 1999]. Conversely, longitudinal studies have failed to find a consistent association between a baseline measurement of viral load and duration of infection, clearance of disease, and subsequent risk of acquisition or progression of disease [Clavel et al., 2000;Crum et al., 2004;van Duin et al., 2002]. The relationship between viral load and disease also varies with HPV type. For example, cross-sectional studies show that HPV16 viral load increases with increasing disease severity, whereas that of HPV18 does not [Ho et al., 2005;Swan et al., 1999]. One study did find an association between high viral load of HPV types 16, 31 and 18/44 and carcinoma in situ, however the association was much stronger in the case of HPV 16, compared to the other types.

The amount of HPV-DNA is a complex sum of number, size, and state of the HPV-associated lesions. Some of the highest viral loads are associated with ultimately resolving CIN-1 and low-grade squamous intraepithelial lesions (LSIL), producing large amounts of virus analogous to benign warts. Cervical cancers do not produce large amounts of intact virus, and this is probably linked to the disruption of the HPV virion that precedes genomic integration [Moscicki et al., 2006]. In addition, HPV 16 is able to induce malignant transformation without integrating into the cell genome in contrast to HPV 18, HPV 31 and HPV 35, which always seem to be present in an integrated physical state in malignant lesions [Hudelist et al., 2004;Pirami et al., 1997], and viral replication is much more productive in episomal form, than integrated.
The influence of multiple HPV-type infection

Cervical cancer is typically a monoclonal event related to a single HPV type. However, the surrounding cervical epithelium can still be infected with other types. As measured by sensitive DNA detection methods, more than 20–30% of women with cervical infections have more than one type, regardless of stage of pathology [Moscicki et al., 2004]. The acquisition is probably through sexual co-transmission. Natural history studies show an increased risk of acquisition of new HPV types in women already infected, compared with those who are HPV negative [Liaw et al., 2001; Rousseau et al., 2001]. For example, the risk of acquiring HPV58 is up to seven times higher in women with an incident HPV16 or HPV18 infection compared with those who are not infected with these types [Mendez et al., 2005]. It is still not clear whether infection with multiple HPV types interferes, either directly or immunologically, with the persistence of a given HPV type or with progression [Castle et al., 2002; Moscicki et al., 2004; Schiffman and Kjaer, 2003]. If co-infection confers some mutual type-specific survival benefit, then the elimination of one HPV type (e.g. through vaccination) could have an unexpected beneficial effect by making it harder for associated types to persist [Woodman et al., 2007].

1.2.4. Immune response against HPV

The predominate natural immune response is characterized by a strong localized cell-mediated immunity (Th1), which is associated with lesion regression and the generation of serum neutralizing antibodies (Th2) [Stanley, 2006]. The immunodominant neutralizing epitopes are located on the major capsid protein L1. However, HPV utilizes several methods to avoid the host immune system [Kanodia et al., 2007; Stanley, 2006]. Basically, HPV keeps itself “invisible” to the host immune system. The essential signals for initiation of an immune response in epithelia are absent [Kupper and Fuhlbrigge, 2004]. The non-lytic nature of the viral life cycle does not trigger dendritic cells, the viral proteins are produced at low concentrations and are not secreted and stay mainly in the nucleus of the cells, the highly immunogenic L proteines are synthesised in the outer layers of the epithelium, the virus uses molecular mimicry (sequence similarity of parts of viral proteins and host
proteins) as shown for HPV 16 E7 [Natale et al., 2000] and there is no blood born phase in the viral life cycle. In addition, HPV is able to downregulate the expression of host interferon genes, with decreased innate and adaptive host immune responsiveness [Daneri-Navarro et al., 2005; Hasan et al., 2007; Stanley, 2006]. There is some evidence to suggest that unique molecular mechanisms allow HPV-16 to evade clearance by the immune system, and so be more likely to persist or take longer to clear than other types [Khammanivong et al., 2003; Molano et al., 2003; Schiffinan et al., 2005; Strickler et al., 2003]. Despite viral immune evasion, the host immune system is able to effectively combat most HPV infections. HR-HPV infections often take longer to clear than LR-HPV infections. The infection becomes persistent only when the immune system fails to eliminate or control HPV. Successful clearance of HPV infections, particularly HR-HPV type-16 and 18, is diminished among immunocompromised individuals [Moscicki, 1999; Strickler et al., 2003]. Among subjects who had an incident infection with HPV-16, a maximum of 57% became seropositive for IgG within 8.3 months and 37% had IgA within 14 months [Ho et al., 2004]. A significantly higher seroprevalence for HPV-16 in women with current HPV-associated lesions or repeated detection of HPV-16 DNA suggests that viral-load and persistence are primary factors for the development of a natural humoral immune response to L1.

1.2.5. Detection of cervical HPV infection

HPV DNA testing

Strains of HPV cannot be grown in conventional culture, and serological assays used for their detection have only limited accuracy [Dillner, 1999]. Therefore, the diagnosis of HPV infection is almost entirely based on the detection of HPV DNA. Currently, human papillomavirus (HPV) DNA tests validated in large trials and epidemiological studies are the hybrid capture second-generation (HC2) HPV DNA assay (Digene Corp. Bethville) and a variety of polymerase chain reaction (PCR) protocols. Ideally, an HPV test should allow detection of multiple HPV types, identify
individual types, and provide quantitative information about the viral load of each individual type found [Iftner and Villa, 2003].

**Hybrid Capture HPV DNA Assay**

HC2 is based on hybridization in solution of long synthetic RNA probes complementary to the genomic sequence of 13 high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and five low-risk (6, 11, 42, 43, 44) HPV types, which are used to prepare high (B) and low (A) probe cocktails that are used in two separate reactions. DNA present in the biological specimen is hybridized in solution with each of the probe cocktails allowing the formation of specific HPV DNA-RNA hybrids. These hybrids are then captured and detected by a series of reactions that give rise to a luminescent product that can be measured in a luminometer, providing a semi-quantitative measure of the viral load. The HC2 is easy to perform in clinical settings, and is suitable for automation. Furthermore, HC2 does not require special facilities to avoid cross-contamination, because it does not rely on target amplification to achieve high sensitivity, as do PCR protocols. Often only the high-risk cocktail is used; this is clinically more relevant and reduces time and cost of the test.

HC2 has some limitations. It distinguishes between a group of 13 HR and 5 LR-HPV types, but does not allow specific individual types of HPV to be identified. The detection limit of 5,000 genome equivalents makes it less sensitive than PCR [Cope et al., 1997; Molijn et al., 2005], and some authors have reported false positive reactions due to cross-reactivity with low-risk types of HPV [Poljak et al., 2002].

**PCR-Based Assays**

HPV DNA can be selectively amplified by a series of reactions that lead to an exponential increase in the viral sequences present in the biological specimen. Analysis of the amplified products can be done in different ways including gel electrophoresis, dot blot or line strip hybridization, and ultimately can be coupled to direct DNA sequencing. The sensitivity and specificity of PCR-based methods can vary, depending mainly on the primer sets, the size of the PCR product, reaction conditions and performance of the DNA polymerase used in the reaction, the
spectrum of HPV DNA amplified and ability to detect multiple types. PCR can theoretically produce one million copies from a single double stranded DNA molecule after 30 cycles of amplification. Therefore, care must be taken to avoid false-positive results derived from cross-contaminated specimens or reagents.

The most widely used protocols employ consensus primers that are directed to a highly conserved region of the L1 gene, and are potentially capable of detecting all mucosal HPV types. Among these are the single pair of consensus primers GP5/6 and its extended version GP5+/6+ and the MY09/11 degenerate primers and its modified version, PGMY09/11.

Full distinction of roughly 40 types can be achieved by hybridization with type-specific probes, that can be performed in different formats, including line strip assays and microtiter plates. The Line Blot Assay (LBA) and Line Probe Assay (LiPA) allow type-specific detection of individual types in one step. Both the PGMY-LBA [Coutlee et al., 1999] and SPF10-LiPA [Kleter et al., 1999] provide a robust detection system combining both the broad-spectrum and type-specific detection systems.

Samples with multiple HPV types.

HC2 does not discriminate between different types in a multiple infection. Conversely, not all PCR-based methods perform equally well in detecting multiple infections because of limitations in the number of HPV types detectable and assay performance. It has been shown for example that the GP5+/6+ system detects only 47% of samples with multiple HPV types in comparison to 90% detected by MY09/11 PCR [Qu et al., 1997]. Another problem is the difference in sensitivity for distinctive HPV types between different test systems used and the reproducibility of different HPV tests for determining the exact HPV type in the sample [Castle et al., 2002; Jacobs et al., 1999]. In general, it seems that PCR systems using multiple primers such as PGMY09/11 and SPF-PCR are more robust to detect multiple infections than systems using single consensus primers such as GP5+/6+. This may especially be true in cases of mixed infections where one type is present in large amounts. The kinetics of the PCR reaction when using single
primer pairs is then unfavorable for types present in smaller amounts. The SPF10-LiPA system is particularly advantageous in clinical, diagnostic uses because the detection system requires a small fragment (65bp) for amplification which has optimal sensitivity in cervical scrapes and even formalin-fixed, paraffin embedded biopsy tissue where the DNA may be degraded, as a result of DNA cross-linking [Kleter et al., 1999; Melchers et al., 1999].
1.2.6. Epidemiology of HPV

Evidence on worldwide prevalence and type-distribution, according to severity of cervical disease has been compiled by IARC meta-analysis, as well as pooled analysis from IARC studies. Overall, it is clear that the prevalence of HPV infection increases as cervical disease state of the group under investigation becomes more severe: 1.4% – 25.6% in cytologically normal women to 87% in women with SCC [Franceschi and Clifford, 2005].

With increasing severity of the cervical lesion, the distribution of HPV types becomes more representative of those HPV types detected in ICC. HPV16 is found in approximately 18.9% of all LSIL cases, 45% of HSIL cases and 55% of ICCs [Smith et al., 2007]. This is clearly demonstrated in Figure 1.9. Of all HR-HPV types, only HPV16 and 18 were found more frequently in SCC than in LSILs, underlining their carcinogenic importance.
In cervical cancer

It is widely accepted that persistent HR HPV infection is a necessary cause of cervical cancer [Walboomers et al., 1999]. An IARC pooled analysis of 3,607 women with incident, histologically confirmed cervical cancer recruited in 25 countries was adapted from Franceschi and Clifford, JNCI 2005.
published in 2004 [Munoz et al., 2004]. A central lab had performed the HPV DNA detection and typing in cervical cells or biopsies using PCR assays. HPV DNA was detected in 96% of specimens, and the 15 most common types were, in descending order of frequency: 16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 39, 51, 73, 68 and 66.

4.4% Of cancers were classified as positive for “HPVX”, but these most likely represent the failed detection of known types rather than infections of yet undiscovered types [Clifford et al., 2006a]. Higher than average proportions of type 16 (67.6%) were found in northern Africa, of type 18 (25.7%) in south Asia, of type 45 (15.0%) in sub-Saharan Africa and of type 31 (7.4%) in Central/South America.

Figure 1.10. Prevalence of HPV types in ICC worldwide.

From: Munoz et al., 2004
A comprehensive meta-analysis of 85 studies published up to February 2002 (including those in the aforementioned IARC series) [Clifford et al., 2003] was recently updated to include more than 14,500 cases of ICC from studies published up to January 2006 [Smith et al., 2007]. The most common HPV types identified were, in order of decreasing prevalence: HPV-16, -18, -33, -45, -31, -52, -35, -59, -56, -51, -39, -6, -68, -73, -66 and -70. The prevalence of high-risk HPV types is shown in Table 1.3.

HPV-16 and -18 accounted for 70% of all cervical cancer cases worldwide, and the eight most common types (HPV-16, -18, -33, -45, -31, -52, -35 and -39) accounted for 90% of cases. Very consistent findings can therefore be seen when comparing the pooled analysis and meta-analysis approaches (Fig. 1.10. and Table 1.3., respectively).
The most common HPV types in cervical cancer from the most recent meta-analysis are shown in Figure 1.12.
Figure 1.12. Type-specific prevalence of human papillomavirus (HPV) infection in (a) invasive cervical cancer (ICC) cases and (b) high-grade squamous epithelial lesions (HSIL), stratified by continent. Adapted from [Smith et al., 2007]
The same eight HPV types (HPV-16, -18, -31, -33, -35, -45, -52, and -58) were most frequent in each region, with the slight possible exception of HPV-56 being the eighth most common type instead of HPV-52 in Europe. HPV-16 prevalence varies from 52% in Asia to 58% in Europe, and HPV-18 prevalence varies from 13% in South/Central America to 22% in North America. The relative importance of HPV types 31, 33, 35, 45, 52 and 58 appeared to differ somewhat by region, with HPV-58 prevalence being particularly high in Asia.

This meta-analysis also showed that HPV type distribution varies significantly between squamous-cell carcinoma (SCC) and adenocarcinoma (ADC), with HPV-16 being identified more often in SCC than in ADC and HPV-18 more in ADC than in SCC.

**In HSIL/CIN3**

The same authors as above also recently updated a comprehensive meta-analysis of IARC [Clifford et al., 2003] to include more than 7000 cases of HSIL from studies published up to January 2006 [Smith et al., 2007]. Overall HPV prevalence in HSIL was 85%, ranging from 78% in Asia to 88% in Europe. Combined HPV16/18 prevalence in all HSIL cases was 52%. Overall, the 8 most common HPV types in HSIL (Table 1.3.; figure 1.12.) were largely similar to those in cervical cancer, except for a noticeable lower rate of HPV45. HPV16 was the predominant type in HSIL from all continents studied, varying from 34% in Asia to 52% in Europe (Figure 1.12.).

**In LSIL**

A comprehensive meta-analysis of 53 studies published up to June 2004 included a total of 8308 LSIL cases (cytologically or histologically diagnosed) [Clifford et al., 2005b]. The majority of cases came from studies in Europe (49%) and North America (29%), with African and Asian studies each representing only 3% of LSIL cases. Overall HPV positivity in LSIL varied from 29 to 100% across the included studies, this despite the fact that only studies using one of four validated broad spectrum PCR primer sets (MY09/11, PGMY09/11, GP5+/6+ or SPF10), were included in this meta-analysis. Variations in both cytological/histological assessment, sensitivity of PCR assays and regional definitions of LSIL are likely to account for these differences.
Among 5910 HPV-positive LSIL, HPV-16 was the most common type (26.6%), followed by HPV-31 (11.7%), -51 (10.9%), -53 (10.1%), -56 (9.7%), -52 (8.8%), -18 (8.6%), -66 (8.5%) and 58 (8.5%). Many other HPV types were also detected in at least 5% of LSIL, thus highlighting the broad heterogeneity of HPV types in LSIL. HPV-16 was the most prevalent HPV type in all regions, although the proportion of HPV-positive LSIL attributable to HPV-16 differed significantly from 16% in Africa to 29% in Europe. The proportion of HPV-positive LSIL attributable to HPV-18 differed significantly from 5% of HPV-positive LSIL in South/Central America to 12% in North America.

**In women with normal cervix**

In 2005, IARC published a pooled analysis of HPV infection in women, randomly selected from the general population of 13 areas from 11 countries (Nigeria, India, Vietnam, Thailand, Korea, Colombia, Argentina, Chile, the Netherlands, Italy, and Spain) including 15 613 women aged 15–74 years without cytological abnormalities [Clifford et al., 2005a]. All HPV testing was by GP5+/6+ PCR-based enzyme immunoassay. The proportion of HPV-positive women infected with different HPV types was compared by study area and between pooled regions with age-adjusted odds ratios (ORs). Age-standardised HPV prevalence varied nearly 20 times between populations, from 1·4% (95% CI 0·5–2·2) in Spain to 25·6% (22·4–28·8) in Nigeria. Although both overall HPV prevalence and HPV16 prevalence were highest in sub-Saharan Africa, HPV-positive women in Europe were significantly more likely to be infected with HPV16 than were those in sub-Saharan Africa (OR 2·64), and were significantly less likely to be infected with high-risk HPV types other than HPV16 (OR 0·57) and/or low-risk HPV types (OR 0·44, p=0·0002). Women from South America had HPV-type distribution in between those from sub-Saharan Africa and Europe. Heterogeneity between areas of Asia was significant.

This heterogeneity in HPV type distribution among women from different populations is an important consideration when developing screening tests for the virus and predicting the effect of vaccines on the incidence of infection.

Large studies in women from the general population, mainly from North America [Burk et al., 1996; Giuliano et al., 2001] and Western Europe [Kjaer et al., 1997; Peto
et al., 2004] showed that the prevalence of HPV was highest in women younger than 25 years of age, corresponding to the onset of exposure through sexual activity, and then steadily declined. Subsequent cohort studies from Costa Rica and Honduras demonstrated a second pattern that shows the first peak of HPV prevalence among women under 25 years of age followed by the expected decline in prevalence until around 45-50 years, but with a subsequent second increase and peak in later years [Castle et al., 2005; Franco et al., 1999; Herrero et al., 2000; Lazcano-Ponce et al., 2001; Munoz et al., 2004; Poljak et al., 2002; Sherman et al., 2003].

A recently published worldwide cross-sectional study demonstrated that the variation in age distribution is even more diverse [Franceschi et al., 2006]. This study included 18,498 sexually active women, aged 15-74 years, from the general population of 15 areas in 4 continents. Similar standardized protocols for women's enrolment, cervical specimen collection and PCR-based assays for HPV testing were used. Age-standardized HPV prevalence varied more than 10-fold between populations, as did the shape of age-specific curves. HPV prevalence peaked below age 25 or 35, and declined with age in Italy, the Netherlands, Spain, Argentina, Korea and in Lampang, Thailand and Ho Chi Minh, Vietnam. This was not the case in Songkla, Thailand nor Hanoi, Vietnam, where HPV prevalence was low in all age groups. In Chile, Colombia and Mexico, a second peak of HPV prevalence was detected among older women. In the poorest study areas in Asia (Shanxi, China and Dindigul, India), and in Nigeria, HPV prevalence was high across all age groups. The substantial differences observed in age-specific curves of HPV prevalence between populations may have a variety of explanations.

The most interesting age pattern that the overview of IARC HPV Prevalence Surveys disclosed, however, is the flat age curve observed in the lowest-income areas of Asia and in Nigeria, where HPV prevalence was similar across age groups. Of the 3 areas that showed high HPV prevalence and no decline in older age groups, all have high rates of cervical cancer incidence and mortality [Ferlay et al., 2005], and very low income levels. Although HPV prevalence is consistently high, the age-specific curve may differ across sub-Saharan African regions [Clifford and Franceschi, 2005]. In studies from South Africa, Tanzania, the Gambia and Senegal, HPV prevalence remained high, or even increased, in middle and old age. Similarly elevated HPV prevalence across all age groups is also typically seen in studies of HPV among HIV-
positive women in different continents [Mayaud et al., 2001; Palefsky et al., 1999], however, the vast majority of women from the Nigerian study could be assumed to be HIV-negative, the proportion of HIV-positive women not exceeding 3% [Thomas et al., 2004].

Figure 1.13. Age-specific HPV distribution for The Netherlands and Nigeria.

Adapted from Franceschi et al., 2006.
1.3. Prevention of cervical cancer

1.3.1. Screening of cervical pre-cancer and cancer

The objective of cervical screening is to prevent invasive cervical cancer by detecting and treating women with CIN 2/3 lesions or improving the prognosis by detecting and treating early stage cancers (downgrading).

The critical components of cervical screening are the following: screening tests of good quality and, in screen-positive women, prompt diagnostic investigations such as colposcopically directed biopsies; appropriate treatment; and post-treatment follow-up to detect persistence or recurrence of disease [Denny and Sankaranarayanan, 2006]. Ensuring high levels of participation, sufficient health care infrastructure and human resources, and linkage of the critical screening components in an organized fashion are essential for the success of screening programs [Alliance for Cervical Cancer Prevention, 2004]. The inability to implement effective cytologic screening and the perceived lack of applicable alternative approaches have delayed the introduction of cervical cancer prevention programs in developing countries by several decades.

Although there have been no randomized trials to evaluate the impact of screening on cervical cancer incidence and mortality, and all data on the effect of screening have come from cohort and case-control studies, a marked reduction in the incidence of and mortality from cervical cancer following the introduction of cytologically based screening programs in a variety of developed countries has been interpreted as strong nonexperimental support for organized cervical cancer screening programs [Hakama, 1986].


As mentioned in a recently published guidelines manual for cervical cancer prevention in settings with limited resources, a good screening test should be:
accurate, reproducible, inexpensive, easy to perform and easy to follow up, acceptable and safe. [WHO, 2006].

*Cytologic screening with conventional Pap smear*

Dr Papanicolaou first published his findings on cancer features in exfoliative cervical cells in 1928. Cells from the transformation zone are collected, using a wooden extended-tip spatula or brush. The cells are fixed on a glass slide and stained, using a special method (papanicolaou staining) in the laboratory. These slides are then read by trained cytologists and quality control is recommended by a cytopathologist, who ideally reexamines all positive slides and 10% of the negatives. Application of this test therefore requires a laboratory infrastructure; trained cytotechnologists and pathologists for processing slides and reporting; internal and external quality control; and a system for communicating the results to the women. High quality training, continuing education, and proficiency testing of personnel are essential to ensure reliable test results. When all these requirements are addressed, cytologic testing has been shown to be moderately sensitive and highly specific in detecting CIN 2/3 lesions. However, in most routine settings, the testing has been shown to be poorly sensitive - with a wide sensitivity range - in detecting cervical neoplasia. In recent reviews, the sensitivity to detect CIN 2/3 lesions ranged from 47% to 62% and the specificity from 60% to 95% [Denny and Sankaranarayanan, 2006]. In several cross-sectional studies from developing countries assessing the accuracy of cytologic screening, the sensitivity varied between 44% and 78% and the specificity between 91% and 96% [Denny and Sankaranarayanan, 2006]. Besides the test characteristics, other issues like the 3 visits required for cytologic screening (testing-, results-, and treatment visit), have major programmatic and logistic challenges.

*Cytologic screening with liquid-based cytology*

With liquid based cytology (LBC), the cells are put directly into a liquid transport medium by the smear taker and are later plated out as a thin-layer of cells on glass in the laboratory. This provides a uniform, more representative thin-layer (almost monolayer) of approximately 80 000 cells in a convenient 13 - 19 mm disk on the slide, which is ideally suited to automated reading, as many of the problems, such as drying artefacts, clumps of cells and obscuring blood and mucus, are removed. The
slide can also be read by a cytologist. LBC with thin-layer or monolayer slide preparation technology has been introduced as a potential solution to concerns about the shortcomings of conventional Papanicolaou (Pap) smears in cervical cancer screening and is now the selected screening method for some developed countries like US, UK and other European and Asian countries. Although in the past decade, several studies have shown the detection rate of LBC for squamous intraepithelial lesions to be equivalent to or greater than that of the conventional Pap smear method with a higher proportion of slides adequate for assessment [Hutchinson et al., 1999; Limaye et al., 2003; Papillo et al., 1998; Weintraub and Morabia, 2000], a recent systematic review could not demonstrate any advantage in higher slide adequacy or sensitivity for HSIL among high-quality studies [Davey et al., 2006]. A recent Liquid-based cytology slides can now be prepared by 2 systems: ThinPrep by Cytyc Corp (Marlborough, Mass) and SurePath, (previously known as AutoCytePrep or CytoRich) by TriPath Imaging (Burlington, NC). The cells remaining in the liquid medium are also suitable for additional testing for the human papillomavirus or other sexually transmitted diseases, as needed.

**HPV testing**

See also section 1.1.5; Detection of cervical HPV infection.

A number of cross-sectional studies have evaluated the use of HPV DNA testing as a primary screening test. These studies have included significant numbers of women and have been conducted in developing countries with a high prevalence of cervical cancer (Mexico [Salmeron et al., 2003], Costa Rica [Schiffman et al., 2000], South Africa [Kuhn et al., 2000] and China [Belinson et al., 1999]. Compared with cytologic evaluation, HPV DNA testing for high-risk types of HPV showed a consistently higher sensitivity (84.9% - 97.6%) for the detection of CIN 2/3 or greater in these studies, but it showed a somewhat lower specificity (81.8% - 94.4%).

A comprehensive meta-analysis comparing primary screening with HC2 and cytology concluded that HC2 generally detects 23% more CIN2+, bus is 6% less specific [Arbyn et al., 2006]. This unprecedented negative predictive value (NPV) remains high prospectively with 5-year disease rates in HPV negative women being equivalent to those of cytology-negative women at 2 years [Bulkmans et al., 2005].
The high NPV for high-risk types of HPV has important implications for screening programs. First, screening intervals may be significantly increased in women older than 30 years who have tested negative for high-risk HPV DNA, as the risk of these women developing cervical cancer over a 5- to 10-year period is negligible [Khan et al., 2005; Villa and Denny, 2006].

Testing for HPV by HC2 has been shown to be more sensitive than repeat cytology or colposcopy in triage of ASC-US smears by the ASCUS-LSIL Triage Study (ALTS), and it was also more sensitive than cytology in a meta-analysis of several comparative studies [Arbyn et al., 2005]. This has led to its introduction in many countries, including the US and France, for the triage of borderline (ASC-US) smear abnormalities.

HPV testing, using self-collected vaginal swabs, has also been proposed as an adjunctive or alternative cervical cancer control measure, especially in developing countries [Ogilvie et al., 2005]. A positive predictive value (PPV) is an important test characteristic, and should be as high as possible because it will decide on the proportion of correct treatments/over-treatment or correct/incorrect colposcopy referrals in a “screen-and-treat” setup, or colposcopy referral setup, respectively. The PPV will depend on the prevalence of HR-HPV in the screened population, as well as with the associations with CIN2+ (associated with persistent infection). Therefore, efficiency of this test will be determined by the age-specific HR-HPV distribution in the population. It has been stated that HR-HPV testing could be used as a screening test in women older that 30-35 years [Cuzick, 1999; Goldie et al., 2005].

Cytologic assessment could be used to triage women who test positive for HPV DNA. Women found to be HPV positive, but with a negative or ASC-US cytologic result, could be safely managed with repeated testing 12 months later [Cuzick et al., 2003]. Owing to the 20% or so greater sensitivity of HPV testing, this approach would improve detection rates of high-grade cervical cancer precursors without increasing the colposcopy referral rate.

In order to provide a simple and affordable HPV test for use in poor-resource countries, PATH launched the Screening Technologies to Advance Rapid Testing (START) project in 2003 in with two different biochemical screening tests are being developed. One is a rapid batch assay to detect the DNA of HR HPV in less than 2 hours with minimal training and equipment and is based upon Hybrid Capture
technology (Digene Corporation). A second assay is a lateral flow strip to detect E6 protein from oncogenic types of HPV in less than 20 minutes (Arbor Vita Corporation; Sunnyvale, CA). Both companies agreed to provide a competitive price for developing countries. (http://path.org/projects/start_project.php; www.hpvtoday.com/_english/_contents/HPVToday_08.pdf).

Visual screening of the cervix

a) Visual Inspection with Acetic Acid

Visual inspection of the cervix after application of a 3% to 5% solution of acetic acid (VIA) - also known as direct visual inspection (DVI), the acetic acid test (AAT), or cervicoscopy - is the most widely evaluated visual screening test. VIA involves naked-eye inspection (no magnification) of the uterine cervix, lit up by a bright torch light or a halogen focus lamp, 1 to 2 minutes after diluted acetic acid has been applied by means of a cotton swab or a spray. A positive VIA test result is characterized by well-defined acetowhite areas close to the squamocolumnar junction (SCJ) or by the white color of a cervical growth or the entire cervix [Sankaranarayanan et al., 2003]. Acetowhiteness is not specific to cervical neoplasia and may occur in immature squamous metaplasia and in inflamed or regenerating cervical epithelium. Acetowhite areas associated with CIN are localized in the transformation zone and are usually well demarcated and dull white, and early cancerous growths turn intensely opaque. Anything that does not meet the criteria of a positive test result, including the absence of acetowhite lesions; faint, ill-defined, translucent acetowhite areas; faint acetowhiteness of endocervical polyps; nabothian cysts; acetowhite dots; and prominent SCJ is categorized as negative.

VIA is described as an easy-to-learn, inexpensive method that requires minimal equipment rather than a laboratory infrastructure and yields real-time results - making it possible to diagnose and treat in the same session. Physicians, nurses, midwives, and paramedical health workers can be rapidly trained to provide VIA in courses of 5 to 10 days [Blumenthal et al., 2005].

The test characteristics of VIA have been evaluated in several cross-sectional studies in developing countries. These studies involving together more than 150,000 women have reported promising results, supporting VIA as an alternative to cervical cytology in these settings. After adjusting for the effects of verification bias, pooled estimates
regarding the sensitivity and specificity of VIA to detect high-grade CIN ranged between 62% and 80% for sensitivity and between 77% and 84% for specificity [Sankaranarayanan et al., 2005].

b) Visual inspection with VIAM (VIA with magnification)
Whether low-level magnification (a magnification of 2 to 4) could improve the diagnostic accuracy of VIA by eliminating a proportion of false-positive appearances due to squamous metaplasia and inflammatory conditions has been investigated in crosssectional studies [Sankaranarayanan et al., 2005]. However, magnification did not improve the test performance above naked-eye viewing and no improvement in the detection rate of high-grade lesions or cancers was observed using magnification.

c) Visual inspection with Lugol’s iodine
Visual inspection with Lugol’s iodine (VILI) involves naked-eye examination of the cervix after application of the iodine solution. The normal columnar epithelium does not change color after iodine application. On the other hand, a healthy mature squamous epithelium is characterized by an abundance of glycogen and turns dark brown or black following application of iodine, whereas abnormal squamous epithelium contains little or no glycogen and takes on a mustard-yellow color. The VILI test results are reported immediately after application of iodine. A positive result is based on the definite appearance of a mustard-yellow area on the cervix close to the SCJ, or the os, or on a cervical growth [Sankaranarayanan et al., 2003].

A recent multicenter study in India and Africa involving approximately 49,000 women concurrently evaluated VIA and VILI by independent providers using a common protocol [Sankaranarayanan et al., 2004]. The pooled sensitivity and specificity to detect high-grade CIN were 92% and 85%, respectively, for VILI vs. 77% and 86% for VIA -indicating a higher sensitivity for VILI but similar specificity for VILI and VIA in this study. In contrast, in a Latin American study involving about 3000 women, VILI was found to have a sensitivity of 53% and a specificity of 78% to detect high-grade CIN [Sarian et al., 2005].
**Screen and treat**

The immediate availability of test results following visual testing has opened up the option of "screen and treat" or "single visit" approach to ensure a high compliance with treatment of screen-positive women [Denny et al., 2005]. In this approach, screen-positive women without clinical evidence of invasive cancer, and satisfying the criteria for ablative therapy, are immediately treated with cryotherapy without confirmatory colposcopic or histologic investigations. The safety, acceptability, and feasibility of the single-visit approach combining VIA and cryotherapy has been demonstrated in a study in rural Thailand, involving 5999 women [Gaffikin et al., 2003].

Recently, a randomized controlled trial involving 6550 women in South Africa reported on the safety and efficacy of 2 screen-and-treat approaches (visual screening followed by cryotherapy or HPV testing followed by cryotherapy) [Denny et al., 2005]. All participants were screened using HPV testing or VIA and then randomized to 1 of 3 groups: cryotherapy for those who had a positive HPV test result; cryotherapy for those who had a positive VIA test result; or delayed treatment regardless of the result of the VIA or HPV test. At 12 months the cumulative detection of CIN 2/3 through colposcopy was 1.4% (95% CI, 0.8---2.0%) in the HPV DNA group, 2.9% (95% CI, 2.1---3.7%) in the VIA group, and 5.4% (95% CI, 4.3---6.5%) in the delayed-treatment. No major complications were reported in this study.

A recently published study from India involved 1879 women in a "see-and-treat" approach using VIA and cryotherapy by nurses. Minor side effects and complications were documented in less than 3% of women and cure rates after 6 months were 81.4% (752 out of 924) for women with CIN 1; 71.4% (55 out of 77) for CIN 2 and 68.0% (17 out of 25) for CIN 3 [Sankaranarayanan et al., 2007].
1.3.2. Vaccination against HPV

Prophylactic HPV Vaccines

Currently, two prophylactic vaccines, based on HPV virus-like particles (VLP), which are generated by the synthesis *in vitro* of L1 proteins, have been developed and tested in extensive clinical trials. One quadrivalent vaccine against HPV6, 11, 16 and 18 (Gardasil, MSD) is commercially available in the United States and in many other Western countries. The other, a bivalent vaccine against HPV16 and 18 (Cervarix, GSK), is licenced in Australia and under evaluation for other countries.

Vaccine efficacy in unexposed young women against high-grade cervical lesions caused by HPV-16 or 18 is very high, 98% during 3 years after vaccination for the quadrivalent vaccine [FUTURE II Study Group, 2007] and 90% for the bivalent vaccine in an interim analysis of a phase III study after a mean follow-up period of 15 months [Paavonen et al., 2007]. The quadrivalent vaccine was also shown to be 100% protective against incident genital warts, vulvar and vaginal intraepithelial neoplasia and cancer, 3 years after vaccination [Garland et al., 2007]. There was no effect on clearance after 6 months or 12 months of HPV-16 or 18 in women who were HPV DNA positive at the moment of vaccination, as shown for the bivalent vaccine [Hildesheim et al., 2007]. Some cross-protective effect of the bivalent HPV16/18 vaccine against incident infection with HPV45 and HPV31 has also been reported [Harper et al., 2006].

The duration of vaccine efficacy is unknown, but antibody levels have shown to stay high for at least 5 years [Villa et al., 2006], as have preventive effects against persistent infection and cervical lesions caused by the HPV types included [Harper et al., 2006]. One study demonstrated the presence of immune memory after a boost dose at 60 months post primary vaccination by the quadrivalent vaccine [Olsson et al., 2007]. Another study estimated the protective effect to last between 12 years and lifelong for the majority of women vaccinated at 16 – 23 years, using data from an HPV 16 VLP vaccine study in different modelling scenario's [Fraser et al., 2007].

As these vaccines are prophylactic, it is assumed that maximum effectiveness will be achieved by administration before exposure to HPV. However, as long as safety in young children and duration of protection is unknown, it seems unwise to vaccinate
small children and therefore, the current recommendation is to vaccinate female adolescents aged 9-12 years [Saslow et al., 2007].

Studies among males 9-15 years have shown immune responses to the quadrivalent vaccine that are similar to those in females [Block et al., 2006], however no information is yet available on the efficacy of HPV vaccines against infection and HPV-related lesions in males. The anatomical characteristics of the male anogenital epithelia may make antibodies against HPV less effective than in the female genital mucosa.

Little is also known about the specificity or broadness of the neutralizing antibody response by prophylactic vaccines, but initial data indicate that antibody responses are neutralizing against a broad range of variants [Pastrana et al., 2005].

**Therapeutic vaccines**

Therapeutic vaccines target already established HPV infections and the associated precancerous or cancerous lesions. This would benefit individuals of a much broader age range than prophylactic vaccine, and this is especially true for people with HIV (PHIV) who often are concurrently infected by both viruses.

Most efforts have focused on eliciting cytotoxic T lymphocyte responses against E6 and E7. Therapeutic vaccines present far more challenges than prophylactic vaccines, and indeed there is nearly no precedent in the cancer field. In respect to HPV, only data from phase I/II clinical trials are available.

So far, the most significant results obtained were with a HPV16 E7 Mycobacterium Bovis heat shock 65 (HSPE7) fusion protein vaccine with 23% complete response in 58 women with CIN3 [http://www.nventacorp.com/news/pr20070305_Einstein_SGO.htm; last accessed 26/03/07]. An MVA E2 recombinant vaccinia virus used in 34 patients with CIN2/3 [Garcia-Hernandez et al., 2006] showed 59% total response. A randomised controlled trial with DNA vaccine encoding fragments from HPV16/18 E6 and E7 (ZYC101a) including 86 vaccinated and 41 control subjects with CIN2/3, found a significantly higher resolution of lesions only in a subgroup of women younger than 25 years (70%) versus a control group (23%) [Garcia et al., 2004] (http://www.mgipharma.com/wt/page/amolimogene). One study used HSPE7 in different dosages in 15 HIV-positive men with high-grade anal intraepithelial (AIN)
lesions, in which five lesions regressed. There were no adverse effects on changes in HIV viral load and CD4/CD8 ratio [Palefsky et al., 2006a].

**HPV vaccines in HIV-infected women**

Prophylactic vaccines work mainly through humoral immunity, which is relatively well preserved in WHIV before severe immunosuppression. Even if there are reasons to expect adequate response to HPV vaccination in women with HIV (WHIV) [Palefsky et al., 2006b], many would not benefit from a prophylactic vaccine as they would have already been infected with the HPV types present in the vaccine. Therefore, the highest benefit could be expected from vaccinating young adolescents in populations with high HIV prevalence, thus also protecting future generations of WHIV.

However, as HPV VLP vaccines are highly immunogenic with elevated antibody levels, but also stimulate innate [Lenz et al., 2005] and cellular immunity, it might be possible that vaccinating PHIV could offer a boosting effect of previously acquired natural immunity [Markowitz et al., 2007; Villa et al., 2006], an effect that has not yet been elucidated even among HIV-negative women. Studies on immunogenicity and clinical effectiveness in PHIV are still needed. Experience with other vaccines is rather encouraging, although increased dosages or altered administration schedules might be required in PHIV. One study with recombinant hepatitis B virus (HBV) vaccine in adult PHIV showed significantly better seroconversion rates for double dose (47% of 98 vaccinated), compared to standard dose (34% of 94). Rates were also significantly higher in participants with CD4 count ≥350/μl [Fonseca et al., 2005].

Addition of an immunostimulating adjuvant to HBV vaccine resulted in 100% seroconversion rate at 12 months in 19 vaccinated adult PHIV under anti-retroviral treatment (ART) [Cooper et al., 2005]. A study with tetanus toxoid booster in 15 HIV-positive children and young adults, all on HAART, demonstrated diminished though sufficiently preserved anamnestic responses [Ching et al., 2007]. The authors suggested that shorter booster intervals might be considered in WHIV.

The safety of VLP vaccines in PHIV is currently being investigated. Concerns are not very severe, however, as these are non-live vaccines and other such vaccines (e.g. against influenza, Pneumococcus, Meningococcus and *Haemophilus influenzae* type b) are already recommended in this population [Kroger et al., 2006]. HIV viremia
could temporarily surge through CD4 cell stimulation, but this might be controlled by HAART [Palefsky et al., 2006b].

As therapeutic vaccines aim to clear present (or latent) HPV infection and HPV-related pre-cancerous and cancerous lesions, they might be more appropriate for PHIV. Therapeutic vaccines against HPV might also remedy the high recurrence rates that are found among PHIV after treatment of intraepithelial lesions. In a mouse model, a therapeutic vaccine against the E7 subunit was more efficacious after removal of most of the tumor tissue [Sin et al., 2006].
1.4. The immunodeficiency virus epidemic in Africa

1.4.1. The HIV epidemic in sub-Saharan Africa

Figure 1.14. Adults and children estimated to be living with HIV, 2006

Adults and children estimated to be living with HIV, 2006

Total: 39.5 (34.1 – 47.1) million


A total of 39.5 million people were living with HIV in 2006, of which 17.7 million (44.8%) were women of 15 years or older.

Sub-Saharan Africa continues to bear the bulk of the global epidemic. Two thirds (an estimated 24.7 million) of all adults and children with HIV globally live in sub-Saharan Africa, with its epicentre in southern Africa (Figure 1.14). Declines in national HIV prevalence are being observed in some sub-Saharan African countries, but such trends are currently neither strong nor widespread enough to diminish the epidemics’ overall impact in this region. Changes in incidence along with rising AIDS mortality have caused global HIV prevalence (the proportion of people living with HIV) to level off (Figure 1.15). However, the numbers of people living with HIV have continued to rise, due to population growth and, more recently, the life-prolonging effects of antiretroviral therapy.
Hardest-hit is southern Africa, where Zimbabwe remains the only country where national adult HIV prevalence has declined. The declining trend appears to be partly
associated with behaviour changes dating back to the mid- to late-1990s. Meanwhile, the HIV epidemics in Mozambique, South Africa and Swaziland continue to grow. In South Africa, which in terms of sheer numbers has one of the world’s largest HIV epidemics, prevalence of HIV among women attending public antenatal clinics was more than one third (35%) higher in 2005 than it had been in 1999. While HIV infection levels among young pregnant women appear to be stabilizing, they continue to increase among older women. The epidemic is having a significant impact. Death rates from natural causes for women aged 25–34 years increased fivefold between 1997 and 2004, and for males aged 30–44 more than doubled. A large part of those increases is due to the AIDS epidemic.

In East Africa, where HIV infection levels have been lower than in the south of the continent, the general trend of a stabilizing or a declining HIV prevalence appears to be continuing. National HIV prevalence among pregnant women has declined in Kenya, as it has in Tanzania and, to a lesser extent, in Rwanda. In many other countries though, discrepant trends are often being found at local levels. Meanwhile, new research indicates a possible erosion of the gains Uganda made against AIDS in the 1990s, and HIV prevalence has again been rising in some rural areas. A sudden increase in infection levels among pregnant women in 2005 in Burundi’s capital, Bujumbura, could reverse the general, post–2000 decline in HIV prevalence in that country. West and Central Africa’s smaller epidemics show divergent trends. There are signs of declining HIV prevalence in urban parts of Burkina Faso, Côte d’Ivoire and Ghana, but in Mali the HIV epidemic appears to be growing.

A recent development in sub-Saharan Africa is the emergence of injecting drug use as a potential factor in the HIV epidemics of several countries, notably those of Kenya and Tanzania (as well as Nigeria and South Africa).

Although antiretroviral therapy was considered out of reach for low-resource regions for a long time, adjusted programs for antiretroviral therapy provision have been put in place like for example, the WHO/UNAIDS “3 by 5” initiative to provide ARV treatment to 3 million people by 2005. Increased resources were made available to countries by the Global Fund to Fight AIDS, TB and Malaria, the World Bank, the United States President’s Emergency Plan for AIDS Relief and other bilateral efforts,
as well as by private foundations and nongovernmental organizations. Through the expanded provision of antiretroviral treatment in recent years (Figure 1.15), an estimated two million life years were gained since 2002 in low- and middle-income countries. In sub-Saharan Africa alone, some 790 000 life years have been gained, the vast majority of them in the past two years of antiretroviral treatment scale-up.

**Figure 1.16. People in sub-Saharan Africa on antiretroviral treatment as percentage of those in need, 2002-2005**


1.4.2. The HIV epidemic in Kenya

Kenya has a generalized HIV epidemic. Between 1.1 million and 1.5 million people were living with HIV in 2005 [UNAIDS, 2007]. There is evidence of a decline in HIV prevalence in recent years: national prevalence among adults dropped from 10% in the late 1990s to about 6% in 2005. The declining trend is largely attributed to behavioural changes resulting from large-scale prevention efforts that began in 2000, including delayed sexual debut, increased condom use and reduced rates of sex with multiple partners [Cheluget et al., 2006]. On the other hand, injecting drug use has been observed as an emerging factor in HIV transmission. The national response to HIV/AIDS is guided by the National HIV/AIDS Strategic Plan 2005–2010 that set targets for progressing towards universal access to HIV prevention, care and treatment, including the provision by 2010 of antiretroviral therapy to 75% of men and 80% of women with advanced HIV infection, and of antiretroviral prophylaxis to 80% of infected pregnant women. A commitment was also made to ensuring that, by the same year, 2 million people, including those most at risk, would have received an HIV test.

The provision of antiretroviral therapy began at five pilot sites during 2001. The provision of antiretroviral therapy was made free of charge in all public health facilities in 2006. By the end of that year it was estimated that some 125 000 people were receiving antiretroviral therapy through public and private facilities, the estimated coverage being 44% (35% vs. 65% for men and women >15 years, respectively). Recently, attention has been focused on expanding the provision of paediatric antiretroviral therapy.

By the end of 2005 there were 759 facilities providing services for prevention of mother to child transmission of HIV (PMTCT) in the public sector (United Nations General Assembly Special Session on HIV/AIDS. Country report, Republic of Kenya, 2005. Accessed at: http://data.unaids.org/pub/Report/2006/2006_country_progress_report_kenya_en.pdf on 2 February 2007.). It was estimated that 20% (17%–23%) of HIV-positive pregnant women received antiretroviral prophylaxis in 2005. Since 2000 there has been a significant expansion in the availability and coverage of testing and counselling services. These services are available both at stand-alone sites and integrated into public health facilities. The number of public sector sites increased from three in 2000 to 650 in 2005. The uptake
of services increased during the same period: 1000 clients sought voluntary
counselling and testing in 2000 and 500 000 did so in 2005. United Nations General
population-based survey conducted in 2003 found that 14% of men and 13% of
women had ever been tested for HIV.

1.4.3. Clinical and immunological classification of HIV / AIDS

The WHO clinical staging system for HIV/AIDS, as developed in 1990, emphasized
the use of clinical parameters to guide clinical decision-making for the management
of HIV/AIDS patients. It was designed for use in resource limited settings where there
was limited access to laboratory services. In response to the changing landscape of
HIV/AIDS, particularly in resource limited settings, and specifically to support scale
up of anti-retroviral treatment, revisions and harmonization of the clinical staging and
case definitions for surveillance were required. For this reason, WHO in collaboration
with CDC, held two expert consultative meetings, in June 2004 in Saas Fe,
Switzerland and in December 2004 in Nairobi, Kenya to review and revise the 1994
WHO clinical staging system and AIDS case definitions. The revisions were designed
to strengthen clinical staging and the AIDS case definitions for both adults and
children, and to simplify and standardize definitions for use by a cross-section of
health providers, program managers and surveillance officers. They were also
intended to harmonize paediatric and adult clinical staging and AIDS case definitions
so as to improve patient management, patient monitoring and surveillance efforts
### Table 1.4. WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection

<table>
<thead>
<tr>
<th>Clinical stage 1: Asymptomatic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td></td>
</tr>
<tr>
<td>Persistent generalized lymphadenopathy</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical stage 2: Mild symptoms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate unexplained weight loss</td>
<td></td>
</tr>
<tr>
<td>(&lt;10% of presumed or measured body weight)</td>
<td></td>
</tr>
<tr>
<td>Recurrent respiratory tract infections: sinusitis, tonsillitis, otitis media and pharyngitis</td>
<td></td>
</tr>
<tr>
<td>Herpes zoster</td>
<td></td>
</tr>
<tr>
<td>Angular cheilitis</td>
<td></td>
</tr>
<tr>
<td>Recurrent oral ulceration</td>
<td></td>
</tr>
<tr>
<td>Papular pruritic eruptions</td>
<td></td>
</tr>
<tr>
<td>Seborrhoeic dermatitis</td>
<td></td>
</tr>
<tr>
<td>Fungal nail infections</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical stage 3: Advanced symptoms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexplained severe weight loss (&gt;10% of presumed or measured body weight)</td>
<td></td>
</tr>
<tr>
<td>Unexplained chronic diarrhoea for longer than one month</td>
<td></td>
</tr>
<tr>
<td>Unexplained persistent fever (above 37.6°C intermittent or constant, for longer than one month)</td>
<td></td>
</tr>
<tr>
<td>Persistent oral candidiasis</td>
<td></td>
</tr>
<tr>
<td>Oral hairy leukoplakia</td>
<td></td>
</tr>
<tr>
<td>Pulmonary tuberculosis (current)</td>
<td></td>
</tr>
<tr>
<td>Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)</td>
<td></td>
</tr>
<tr>
<td>Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis</td>
<td></td>
</tr>
<tr>
<td>Unexplained anaemia (&lt;9 g/dl), neutropaenia (&lt;0.5 × 10⁹ per litre)</td>
<td></td>
</tr>
<tr>
<td>or chronic thrombocytopaenia (&lt;50 × 10⁹ per litre)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical stage 4: Severe symptoms</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV wasting syndrome</td>
<td></td>
</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td></td>
</tr>
<tr>
<td>Recurrent severe bacterial pneumonia</td>
<td></td>
</tr>
<tr>
<td>Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month’s duration or visceral at any site)</td>
<td></td>
</tr>
<tr>
<td>Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)</td>
<td></td>
</tr>
<tr>
<td>Extrapulmonary tuberculosis</td>
<td></td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus infection (retinitis or infection of other organs)</td>
<td></td>
</tr>
<tr>
<td>Central nervous system toxoplasmosis</td>
<td></td>
</tr>
<tr>
<td>HIV encephalopathy</td>
<td></td>
</tr>
<tr>
<td>Extrapulmonary cryptococcosis including meningitis</td>
<td></td>
</tr>
<tr>
<td>Disseminated non-tuberculous mycobacterial infection</td>
<td></td>
</tr>
</tbody>
</table>
- Progressive multifocal leukoencephalopathy
- Chronic cryptosporidiosis (with diarrhoea)
- Chronic isosporiasis
- Disseminated mycosis (coccidiomycosis or histoplasmosis)
- Recurrent non-typhoidal Salmonella bacteremia
- Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours
- Invasive cervical carcinoma
- Atypical disseminated leishmaniasis
- Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

<table>
<thead>
<tr>
<th>HIV-associated immunodeficiency</th>
<th>CD4 values: absolute number per mm or %CD4 +</th>
</tr>
</thead>
<tbody>
<tr>
<td>None or not significant</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Mild</td>
<td>350–499</td>
</tr>
<tr>
<td>Advanced</td>
<td>200–349</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;200 or &lt;15%</td>
</tr>
</tbody>
</table>
1.4.4. Antiretroviral treatment

The WHO guidelines development group recommends to start ART in adults and adolescents, depending on either clinical stage (> stage 3) or CD4 counts (< 350), if available (See Table 1.6.).

**Table 1.6. Recommendations for initiating ART in adults and adolescents in accordance with clinical stages and the availability of immunological markers**

<table>
<thead>
<tr>
<th>WHO clinical staging</th>
<th>CD4 testing not Available</th>
<th>CD4 testing available</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Do not treat</td>
<td>Treat if CD4 count is below 200 cells/mm³&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2 Do not treat&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Consider treatment if CD4 count is below 350 cells/mm³ and initiate ART before CD4 count drops below 200 cells/mm³&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3 Treat</td>
<td>Treat irrespective of CD4 cell count</td>
<td></td>
</tr>
<tr>
<td>4 Treat</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> CD4 cell count advisable to assist with determining need for immediate therapy for situations such as pulmonary TB and severe bacterial infections, which may occur at any CD4 level.

<sup>b</sup> A total lymphocyte count of 1200/mm³ or less can be substituted for the CD4 count when the latter is unavailable and mild HIV disease exists. It is not useful in asymptomatic patients. Thus, in the absence of CD4 cell counts and TLCs, patients with WHO adult clinical stage 2 should not be treated.

<sup>c</sup> The initiation of ART is recommended in all HIV-infected pregnant women with WHO clinical stage 3 disease and CD4 counts below 350 cells/mm³.

<sup>d</sup> The initiation of ART is recommended for all HIV-infected patients with CD4 counts below 350 cells/mm³ and pulmonary TB or severe bacterial infection.

<sup>e</sup> The precise CD4 cell level above 200/mm³ at which ARV treatment should be started has not been established.

The WHO “3-by-5 Plan” (Treat 3 million by 2005) recommends that ARV treatment be standardized in resource-limited settings [WHO, 2004]. In particular, it is suggested that countries select a first-line regimen and a limited number of second-line regimens, recognizing that individuals who cannot tolerate or fail the first-line and second-line regimens will be referred for individualized care by specialist physicians.

First-line regimen for adults and adolescents contain two nucleoside reverse transcriptase inhibitors (NRTI) plus one non-NRTI (NNRTI) (Figure 1.17). Regimens
based on combination of two NRTIs plus one NNRTI are efficacious, are generally less expensive than other regimens, have generic formulations, are often available as fixed-dose combinations (FDC) and do not require a cold chain. In addition, they preserve a potent new class (protease inhibitors) for second-line treatments.

The preferred NRTI backbone is composed of zidovudin (AZT) or tenofovir (TDF) combined with either lamivudine (3TC) or emtricitabine (FTC). An NNRTI, either efavirenz (EFV) or nevirapine (NVP), should be added.

A triple NRTI regimen can be considered as an alternative for first-line ART in situations where NNRTI options provide additional complications and to preserve the protease inhibitor class for second-line treatment (e.g. in women with CD4 counts of 250–350 cells/mm³; coinfection with viral hepatitis or tuberculosis; severe adverse reactions to NVP or EFV, infection with HIV-2).

**Fig. 1.17. First-line ARV drugs for adults and adolescents**

![Diagram](image)

1 Preferrable two NRTIs/NNRTI approach is based upon a combination of three drugs: two NRTIs combined with either NVP or EFV as the NNRTI.
2 Preferred NRTI to be combined with 3TC or FTC in standard first-line regimens.
3 Triple NRTI approach (i.e. three NRTI drugs selected only from the options shown within the dotted circle) can be considered as an alternative for first-line regimens in situations where NNRTI options provide additional complications (e.g. women who have CD4 counts between 250 and 350 cells/mm³; viral hepatitis coinfection, severe reactions to NVP or EFV, and HIV-2 infection).

Antiretroviral agents are responsible for a broad range of toxicities, ranging from low-grade intolerances that may be self-limiting to life-threatening side-effects. It may be necessary to change ART because of either toxicity or treatment failure.

Toxicity is related to the inability to tolerate the side-effects of medication and to the significant organ dysfunction that may result. This can be monitored clinically on the
basis of patient reporting and physical examination, and there may also be a limited
number of laboratory tests, depending on the specific combination regimen that is
utilized and the health care setting.

If a change in regimen is needed because of treatment failure, a new second-line
regimen becomes necessary. When the toxicity is related to an identifiable drug in the
regimen, the offending drug can be replaced with another drug that does not have the
same side-effects, e.g. substitution of stavudine (d4T) for ZDV (for anaemia) or NVP
for EFV (for CNS toxicity or pregnancy). Given the limited number of ARV
combination options available in resource-limited settings, it is preferable to pursue
drug substitutions where feasible so that premature switching to completely new
alternative regimens is minimized. Treatment failure can be defined clinically as
assessed by disease progression, immunologically using measurement of the CD4
counts, and/or virologically by measuring viral loads.

Fig. 1.18. Recommended second-line regimens in adults and adolescents in the
event of treatmen failure of first-line ARV regimens

<table>
<thead>
<tr>
<th>For failure on:</th>
<th>Change to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>D4T or ZDV</td>
<td>TDF or ABC</td>
</tr>
<tr>
<td>+ 3TC</td>
<td>+ DdI</td>
</tr>
<tr>
<td>+ NVP or EFV</td>
<td>+ NFV</td>
</tr>
</tbody>
</table>

WHO recommends that in resource-limited settings the basic clinical assessment
before the initiation of ART include documentation of past medical history,
identification of current and past HIV-related illnesses, identification of coexisting
medical conditions that may influence the timing of initiation and choice of ART
(such as TB or pregnancy), and current symptoms and physical signs.

In order to facilitate the scale-up of ARV use in resource-limited settings, WHO has
tiered its monitoring recommendations to primary health care centres (level 1: e.g.
haemoglobin, pregnancy testing or referral for sputum smear), district hospitals
(level 2: added are e.g. full blood count, CD4 cell count, liver tests) and regional
referral centres (level 3: added tests: full serum chemistries, viral load testing).
WHO recognizes the importance of laboratory monitoring for efficacy and safety but does not want restricted infrastructure for these tests to place undue limitations on the scale-up effort [WHO, 2004].

One of the many challenges of the public health approach in challenged limited-resource conditions is to identify and train the necessary health care providers to skilfully administer and monitor ART in PHIV who need it [Van Damme et al., 2006].
1.5. HIV, HPV and cervical dysplasia and cancer in the era of HAART


Hugo De Vuyst, MD1, Flavia Lillo, MD2, Nathalie Broutet, MD, PhD3, and Jennifer S. Smith MPH, PhD4

1Department of Obstetrics and Gynaecology, Ghent University, Belgium; and International Agency for Research on Cancer, World Health Organization, Lyon, France

2Laboratory of Virology, AIDS Center "San Luigi", IRCCS Hospital San Raffaele – Milan, Italy

3Reproductive Health and Research Department, World Health Organization, Geneva, Switzerland

4Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

The references used in this paper are listed at the end of this section
ABSTRACT

OBJECTIVES: To review the literature on the epidemiological association between human papillomavirus (HPV), HIV and cervical neoplasia, and the impact of highly active antiretroviral therapy (HAART) on this association.

METHODS: A review of the literature was conducted by searching MEDLINE using the terms "human papillomavirus", "HPV", "HIV", "cervix", "neoplasm" and "antiretroviral" to identify articles published before December 2006.

RESULTS: HIV-related immunosuppression was strongly associated with a higher prevalence, incidence and persistence of HPV infection and correlated prevalence, incidence, persistence and progression of squamous intra-epithelial lesions (SIL). The association between HIV and invasive cervical cancer has been more difficult to establish, but is now fully recognized. HAART seems to have little, if any, beneficial effect on the natural history of SIL.

CONCLUSIONS: Despite the limited effect of HAART on HPV-related cervical disease, it does increase the life expectancy of HIV-positive women. Therefore, it remains important to closely monitor HPV-related disease in women with HIV and receiving HAART, particularly in those regions of the world where cervical screening is not routinely available.

KEYWORDS
Human papillomavirus, human immunodeficiency virus, cervical neoplasia, highly active antiretroviral therapy
INTRODUCTION

Cancer of the cervix uteri is the second most frequent cancer in women worldwide, with an estimated incidence of 493,000 and mortality of 273,000. Approximately 83% of cases occur in less-developed countries where cervical cancer is the leading cause of cancer death in women (Ferlay et al., 2005).

An estimated 39.5 million people worldwide were living with human immunodeficiency virus (HIV) at the end of 2006, of which two thirds reside in sub-Saharan Africa (UNAIDS, 2006). HIV, human papillomavirus (HPV) and cervical cancer are epidemiologically associated with one another. Opportunistic infections and cancers like cervical cancer are symptoms of acquired immunodeficiency syndrome (AIDS), due to a reduction in the number of CD4 T-helper lymphocytes and impairment in T-cell functions caused by HIV. Invasive cervical carcinoma (ICC) was classified as an AIDS-defining illness in 1993 by the United States Centers for Disease Control and Prevention (CDC) after evidence of a higher prevalence of cervical squamous intraepithelial lesions (SIL) in HIV positive immunosuppressed women (Centers for Disease Control, 1992).

The introduction of highly active antiretroviral therapy (HAART) has significantly improved morbidity and mortality of patients with HIV infection, due largely to immune reconstitution of the host. Although antiretroviral therapy was considered out of reach for low-resource regions for a long time, adjusted programs for antiretroviral therapy provision have been put in place and by the end of 2006, an estimated 28% of people living with HIV and needing antiretroviral therapy treatment in low- and middle- income countries had access (WHO, 2007).

The aim of this work is to review the current knowledge on HIV, HPV and cervical neoplasia/cancer and the effect of HAART on HPV natural history, cervical dysplasia and cancer in women with HIV infection (WHIV).

METHODS

MEDLINE was searched using the terms "human papillomavirus", "HPV", "HIV", "cervix", "neoplasm" and "antiretroviral" to retrieve articles published before December 2006. Additional reports were also obtained by reviewing the reference sections of obtained papers.
RESULTS

The association between HIV and HPV infection

High-risk HPV infection is the central cause for cervical cancer (Bosch et al., 1995) and certain types (i.e., HPV16, 18, 31, 33, 45, 51, 52 and 58) are associated with a greater risk of ICC and its related precursors (Munoz et al., 1992; IARC, 1995).

Prevalence and incidence of HPV in women with HIV

Most cross-sectional studies indicate that the prevalence of cervical HPV DNA is higher in HIV-positive than HIV-negative women, even after controlling for potential confounding factors such as age and sexual behaviour (Sun et al., 1995; Temmerman et al., 1999; Strickler et al., 2005). Table 1 shows that the ratio’s of HPV prevalence between HIV-positive and HIV-negative women was consistently over 1 in the United States, ranging from 1.4 in adolescents to 2.7 in high-risk women in Maryland. Respective HPV prevalence ratios in other American countries ranged from 1.5 in Brazil to 3.2 in Honduras; in Europe from 1.1 in Austria to 9.3 in Italy; and in Africa from 1.0 in Tanzania to 3.6 in Senegal. Given that both HIV and HPV are sexually transmitted and thus share some risk factors, these positive associations found in the majority of available cross-sectional data were not unexpected.

Cohort studies provided the most persuasive evidence that the risk of acquiring new (incident) cervical HPV infections may be higher in HIV-positive than HIV-negative women (Sun et al., 1997; Minkoff et al., 1998; Silverberg et al., 2002; Chaturvedi et al., 2005; Strickler et al., 2005). However, the majority of these studies did not adequately control for differences in sexual behaviour between women with and without HIV in order to determine whether these two infections are simply transmitted sexually at the same time, or whether WHIV are truly at a higher risk of HPV acquisition.

Impact of HIV on HPV persistence

Persistent HPV infection significantly increases the risk of progression to cervical dysplasia (Remmink et al., 1995; Walboomers et al., 1999; Kjaer et al., 2002). Factors mediating HPV persistence in WHIV, however, are not well
understood. Although data are limited, it has been demonstrated that WHIV have a higher risk of developing persistent HPV infections than HIV-negative women. This has been shown for persistence of any HPV type (persistence of 24% in HIV-positive vs. 4% in HIV-negative women, Ahdieh et al., 2000; and 61% vs. 23%, Sun et al., 1997, respectively), as well as for persistence of HPV types 16 or 18 (13% vs. 2%, Ellerbrock et al., 2000, respectively), as for persistence of any high-risk types (Minkoff et al., 1998).

**Impact of HIV on the range of HPV types**

Several studies have found a broader range of both high-risk- low-risk HPV types in HIV-positive compared to HIV-negative women (Goncalves et al., 1999; Palefsky et al., 1999; Ellerbrock et al., 2000). Others, however, have reported no difference (Jamieson et al., 2002). Types which were identified more frequently include HPV35, 44, 54, 59, 66 (Cappiello et al., 1997), and HPV11, 39, 43, 51 and 59 in Zimbabwe (Baay et al., 2004). Broker et al. (2001) compared HIV-positive with HIV-negative women who were immunosuppressed due to renal failure. In the group of WHIV, a higher prevalence of non-oncogenic types from the ‘novel’ A3 phylogenetic subgroup (HPV61, 62, 72, 81, 83 and 84) was found.

In a recent meta-analysis of 20 studies including 5,578 WHIV worldwide (Clifford et al., 2006), the most striking finding was that WHIV with HSIL had an increased risk of prevalent multiple-type HPV infections (OR: 9.3, CI: 6.9-12), compared to women with HSIL from the general population. Whether multiple type infections pose a higher risk for cervical intraepithelial neoplasia (CIN) or ICC than single-type infections is still under debate (Ho et al., 1998; Levi et al., 2002; Cuschieri et al., 2004; Herrero et al., 2005; Trottier et al., 2006). Furthermore, HPV16 was less frequently associated with high-grade SIL (HSIL) in WHIV, compared with women from the general population (Clifford et al., 2006). These results were consistent with previously reported results among women with normal and abnormal cytology in the WIHS and HERS cohorts from the United States (Strickler et al., 2003). The HERS study also showed that HPV16 was more weakly associated with immune status than the other HPV types, suggesting that HPV16 may be better at avoiding the effects of immune surveillance, even among immunocompetent women.
In order to draw conclusions from these HPV typing data, comparable PCR primer sets need to be used to enable identification of the same range of HPV types. Further systematic work is needed in order to compare the distribution of different HPV types in WHIV and HIV-negative women, and on differences between women infected with HIV type-1 and HIV type-2 infections.

**Impact of HIV on HPV Viral load**
Although relatively little data are available on the effect of HIV infection on HPV viral load, most studies show a positive association (Serwadda et al., 1999; Jamieson et al., 2002). HPV viral load was increased in HIV-positive compared to HIV-negative women, among women with normal cytology (Womack et al., 2000), HSIL (Weissenborn et al., 2003) and women with HSIL and HPV-16 infection (Lefevre et al., 2004).

Further data are needed to determine whether HPV viral loads are consistently higher in WHIV, and whether this might explain the higher risk of SIL in these women (Delmas et al., 2000; Duerr et al., 2001; Lillo et al., 2005).

**Role of HIV-related immunosuppression in HPV infection**
Among WHIV, several studies have consistently shown a higher prevalence of any HPV DNA (Palefsky et al., 1999; Jamieson et al., 2002; Strickler et al., 2005), or high-risk HPV types (16, 18, 31, 33, 35 and 45) specifically, as well as more persistent HPV infections (Sun et al., 1997; Ahdieh et al., 2000; Lillo et al., 2001) in women with CD4 <200 cells/mm³. Plasma HIV RNA level and CD4 counts in combination appear to have a stronger association with HPV incidence than with HPV persistence (Strickler et al., 2005). In a modeling study, Goldie et al (2001) estimated the prevalence of transient and persistent HPV infections, stratified by CD4. For women with CD4 counts of <200/mm³, 200-500/mm³, >500/mm³, the estimated prevalence of transient HPV infections was 70%, 56%, and 43%, respectively, and that of persistent infections was 33%, 24%, and 19%. Women with CD4 counts <200 had a highest estimated prevalence of both transient and persistent HPV infections. A study by Broker et al (2001) concluded that HPV infections are very common and are normally held in a sub-clinical (latent) state by a functional immune system and
that these infections can be reactivated under immunosuppressive conditions, a finding also suggested by Strickler et al (2005).

**Other possible mechanisms**

Although there is no great support from available data, HIV could impact on HPV natural history through other mechanisms besides the increased immune-escape pathway in immunosuppressed women. HIV may have a direct viral-viral interaction with HPV, given that both viruses infect macrophages (Clarke and Chetty, 2002). In vitro studies have indicated that expression of HIV tat protein may increase the expression of HPV E1 and L1 viral genes (Dolei et al., 1999) and E7 HPV16 transcription (Vernon et al., 1993). It is uncertain, however, whether HIV may increase the risk of HPV replication or transcription in vivo. Alternatively, inflammatory responses induced by HIV may interfere with a woman’s ability to mount an effective immune response to HPV infection, leading to the development of more persistent HPV infections.

**The association between HIV and cervical dysplasia**

Two terminologies are used to determine stages of cervical neoplasia. Cytological results are reported using the Bethesda nomenclature: low- and high-grade SIL (Kurman and Solomon, 1994); histological results are reported by the International Federation of Gynecology and Obstetrics CIN1-2-3 classification system.

**Prevalence and incidence of squamous intraepithelial lesions in HIV positive women**

SIL have been found to be consistently more prevalent in HIV-positive, compared to HIV-negative women. Wright et al (1994) reported CIN prevalences of 20.0% and 4.2% in HIV-positive and HIV-negative women, respectively. Other studies have found CIN prevalence rates in the order of 26.5% and 7.5% (Six et al., 1998) and 16.7% and 3.5% (Massad et al., 1999), respectively. Table 2 summarises results of comparative studies of low- and high-grade SIL. Studies in WHIV report prevalences of LSIL and HSIL as 28.6% and 14%, respectively in the United Kingdom (Olaitan et al., 1997), 15.3% and 8.1% in the United States (Maiman et al.,
1998) and 21.0% and 2.8% in a European cohort (Delmas et al., 2000). Associations of similar magnitudes have been found in African settings.

Cohort studies reporting on the incidence of SIL shed light not only on the prevalence and incidence of SIL, but also on potential differences in regression and progression rates of SIL. These studies consistently show an increased incidence and progression rate of SIL in HIV-positive compared to HIV-negative women (Table 3). To our knowledge, few cohort data in WHIV from Africa are available. A study from the Cote d’Ivoire describes the evolution of 94 low-grade SIL over a median follow-up time of 5 months. There was a 4.3-fold higher rate of LSIL persistence in HIV-positive, compared to HIV-negative women (76% vs. 17.9%, La Ruche et al., 1999). A cohort study in Senegal found an increased risk of development of high-grade SIL in women with HIV-1 and, to a lesser degree, HIV-2. However, after adjustment for high-risk HPV types and persistence, infection with HIV was no longer a risk factor for development of high-grade SIL (Hawes et al., 2006).

Progression and regression of pre-cancerous lesions in WHIV

Studies have consistently shown increased progression of SIL in HIV-positive, compared to HIV-negative women. Six et al (1998) reported progression of 38.1% of WHIV with LSIL, whereas none of the HIV-negative women progressed over a one year follow-up period. Massad et al (2001) showed greater LSIL progression rates within 6 months after diagnosis among HIV-positive compared to HIV-negative women (13.6% versus 6.8%) and reduced regression rates (43.3% vs. 66.2%), respectively. In contrast, Robinson et al (2002) found no differences in progression or regression of LSIL for WHIV, the majority of whom were on antiretroviral treatment.

Role of immunosuppression in SIL

The correlation between HIV-associated immunosuppression and a higher prevalence of SIL has been described as early as 1991 in a group of HIV-infected and allograft recipients (Vermund et al., 1991). Subsequently, the incidence of SIL has also been shown to be higher in women with lower CD4 counts (Delmas et al., 2000; Massad et al., 2001; Schuman et al., 2003; Strickler et al., 2005). A similar correlation was seen between low CD4 count and progression of cervical lesions (Six et al., 1998; Delmas et al., 2000; Schuman et al., 2003). In the WIHS study, a higher incidence of
any SIL among WHIV with CD4 counts <500 was reported, compared to women with CD4 counts >500. Incidence rates in less-immunocompromised women (CD4 counts >500) appeared similar to HIV-negative women (Harris et al., 2005).

Two studies assessed the association between incidence or progression of SIL and high HIV viral load. Massad et al (2001) report an increased incidence and progression of SIL in women with high HIV viral load. However, Schuman et al (2003) did not confirm this finding. Massad et al (2001) also found HIV viral load to be independently associated with a higher progression of SIL, suggesting a possible HPV-HIV viral interaction, where the level of HIV-associated immunosuppression, through low CD4 count, would determine SIL incidence and regression.

**Risk factors for SIL in WHIV**

Most behavioural risk factors such as number of sexual partners, age at sexual debut, parity, and history of sexually transmitted infections, act as co-factors in the presence of HPV infection. Therefore, in multivariate analysis, it is often (persistent) HPV infection that stands out as the sole or most important independent risk factor for SIL (Maiman et al., 1998; Delmas et al., 2000; Hawes et al., 2006). In studies comparing HIV-positive and HIV-negative women, HIV also remains an independent risk factor for SIL after controlling for HPV infection (Wright, Jr. et al., 1994; Six et al., 1998; Massad et al., 2001; Temmerman et al., 1999). A meta-analysis of 15 studies (1986-1998) by Mandelblatt et al (1999) found that although the association between HPV and SIL was the strongest (OR, 5.10), HIV likely played an important role in SIL development (OR, 1.24) by interacting as a definitive co-factor with HPV (P=0.01).

**The association between HIV and invasive cervical cancer**

*Prevalence and incidence of ICC in WHIV*

A 1996 study by the International Agency for Research on Cancer (IARC) on the relationship between HIV and cervical cancer concluded that there was no evidence of a significant increase in the incidence of cervical cancer among WHIV (IARC, 1996). However, as shown in Table 4, there are now many studies showing an increased risk of cervical cancer especially in developed countries where endemic
rates of cervical cancer are relatively low and where women have better access to care and longer survival following HIV infection. Frisch et al (2000) showed a relative risk (RR) of 5.4 for ICC among WHIV in the United States compared to the general population in the period 1995 to 1998 with a total of 51,760 WHIV included. In Italy, Franceschi et al (1998) and Dal Maso et al (2003) found evidence of a higher risk of ICC among WHIV and reported an increased standardised incidence ratio (SIR) of 21.8 for ICC in WHIV compared to the general population. A similarly increased SIR of 12.8 was observed in a cohort of women from Italy and France (Serraino et al., 1999). One study showed that WHIV had more advanced stage ICC disease than HIV-negative women (OR 3.1). However, in the multiple logistic regression analysis, only symptom duration and lack of a recent pap smear were significant risk factors for advanced ICC disease, and thus results were similar for HIV-positive and HIV-negative women (Fruchter et al., 1998).

In Africa, two population based cancer registries from Uganda (Parkin et al., 1999) and Zimbabwe (Chokunonga et al., 1999) have investigated changes in the incidence of AIDS-related cancer over time, as HIV prevalence has increased in both populations. The registries were examined over the periods 1960-1995 in Uganda and 1990-1995 in Zimbabwe. From these registries, background population-based HIV prevalence in the adult population rose from 0% to 30% in Uganda, and up to 25% by 1997 in Zimbabwe. Both surveys concluded that some AIDS-related cancers did rise over time: Kaposi sarcoma, squamous cell carcinoma of the conjunctiva, and non-Hodgkin lymphoma, however this was not seen for ICC. In both countries, there were no temporal changes in the mean age of ICC presentation. Only recently, Mbulaiteye et al (2006) reported in a record-linkage study from Uganda an increased SIR of 2.4 for cervical cancer. In other African countries, data from South Africa showed an increased risk of cervical cancer in WHIV (Sitas et al., 2000), whereas this was not seen in a Kenyan study (Gichangi et al., 2002). The Kenyan study, however, did report that on average, age at presentation of ICC was 5 years earlier in WHIV compared to HIV-negative women. A similar study from Durban, South Africa reported a quadrupling of HIV seroprevalence among women with ICC over the period 1990 to 1999, with a background increase of adult population HIV seroprevalence from 1.6% to 32.5% (Moodley et al., 2001). The mean age of WHIV with ICC was also 15 years younger. A case-control study in Nairobi reported a
higher HIV prevalence among women with ICC compared to women with uterine fibroids, particularly in the younger age groups (<35 years, Gichangi et al., 2003). It is noteworthy that WHIV with ICC included in this study also had relatively high CD4 counts (only 17% had CD4 <200).

Thus, we may conclude that the association between HIV and ICC has also finally been established for developing countries. The earlier apparently lack of association might have been due to the lack of ART, which inhibited women to survive long enough to develop an invasive carcinoma.

Effect of HAART on HPV natural history, cervical dysplasia and cancer

The mortality rate and life expectancy of HIV infected patients changed significantly since the introduction of HAART, which effectively improved their immunological and virological settings and determined a consistent reduction of most of the HIV-related opportunistic diseases, including some tumors like Kaposi’s sarcoma and non-Hodgkin lymphoma (International Collaboration on HIV and Cancer, 2000).

The effect of HAART on HPV infection

Studies show that cervical HPV infection persists in a high proportion of patients receiving HAART (Heard et al., 1998; Lillo et al., 2001; Chin-Hong and Palefsky, 2002; Conley et al., 2002; de Sanjosé and Palefsky, 2002). Although potent anti-HIV regimens have a clear effect on restitution of the patient’s immune capacity in terms of increase in CD4 counts, this does not seem sufficient to influence high-risk HPV persistence.

Effect of HAART on cervical dysplasia

The effect of antiretroviral treatment on cervical neoplasia is still debated because a clear trend toward a reduction or increase in the prevalence of cervical neoplasia with use of HAART or other antiretroviral therapy has not been found. One of the earliest studies on this topic described the early regression of cervical lesions in women receiving protease inhibitors therapy after a 5 months follow-up period (Heard et al., 1998). More recently, the same group confirmed a higher regression rate of CIN in HAART treated women (Heard et al., 2002). The WIHS cohort study showed that
WHIV on HAART were 40% more likely to have SIL regression and less likely to have SIL progression (Minkoff et al., 2001). The two latter studies showed HAART to be independently associated with CIN regression.

In contrast, Ellerbrock et al (2000) showed increased incidence of CIN among HIV-positive, compared to HIV-negative women (8.3 vs 1.8 cases per 100 person-years) during an observation period of 30 months, but did not observe differences between women untreated or treated with different antiretroviral treatment medication (monotherapy, or a combination).

A longitudinal study with a mean follow up of 15.4 months, reported on SIL outcomes in women untreated or treated with different antiretroviral regimens (Lillo et al., 2001). HAART-treated patients showed the best recovery in terms of HIV viremia suppression and CD4 cell count (mean increase 88 ±17.9 cells/µl). The latter was also described by Moore at al (2002). However, no beneficial effect of HAART was found on incident cases of SIL or on the progression/regression rate of lesions after adjusting for CD4 cell count (Lillo et al., 2001). In a subsequent study (Uberti-Foppa et al., 2003), the former group demonstrated that, after a median follow-up period of 36.4 months, only clinically stable patients (with or without treatment) showed a reduction in the prevalence or progression of cervical lesions when compared to patients with clinically worsening HIV diseases.

**Effect of HAART on invasive cervical cancer**

Data concerning the impact of HAART treatment on the incidence of ICC are still incomplete and controversial. One large study compiled cervical cancer data from 23 studies including 47,936 HIV positive individuals conducted in North America, Europe, and Australia from 1992 to 1999 and concluded that there was no significant change in the incidence rate of cervical cancer during this period (pre- and post-HAART, International Collaboration on HIV and Cancer, 2000). However, the authors commented that the evaluation was hampered by a small number of incident ICC in the cohorts and that a longer follow-up time was needed.
CONCLUSION

There is overwhelming evidence of an association between HIV-related immunosuppression and a higher prevalence, incidence and persistence of HPV infection and correlated prevalence, incidence, persistence and progression of SIL in women. Although it was more difficult to establish, the association between HIV and HPV infection is now fully recognized, also for developing countries. The progression of high-grade SIL to ICC seems less dependent on immunosuppression, in contrast to the development of lower grades of cervical neoplasia (Dal Maso et al., 2001; Palefsky and Holly, 2003). HAART also appears to have little, if any, beneficial effect on the evolution of SIL. However, HAART does prolong the life expectancy of WHIV, hence increasing the opportunity for persistent high-risk HPV infection to cause ICC over time (Dal Maso et al., 2001; Schuman et al., 2003; Heard et al., 2004; Strickler et al., 2005; Mbulaiteye et al., 2006). Prevention of ICC by cervical screening will therefore be even more relevant to WHIV using HAART (Franceschi and Jaffe, in press). To evaluate the effect of maturation of the HIV epidemic on incidence of ICC, cohorts of WHIV on HAART should be closely monitored, particularly in regions of the world where cervical screening is not readily available.
REFERENCES


The impact of HIV infection and immunodeficiency on human papillomavirus type 6 or 11 infection and on genital warts. *Sex Transm Dis* **29**: 427-35.


Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. *J Natl Cancer Inst* **97**: 577-86.


immunodeficiency virus-positive women with high-grade cervical lesions are strongly elevated. *J Clin Microbiol* **41**: 2763-7.


Table 1. Human papillomavirus (HPV)-DNA prevalence in HIV-positive and HIV-negative women

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>Population source</th>
<th>Sample size</th>
<th>Overall HPV prevalence in HIV+ (%)</th>
<th>Overall HPV prevalence in HIV- (%)</th>
<th>HPV prevalence ratio (HIV+/HIV-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>United States</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watts et al (2005)</td>
<td>WHS study (6 sites)</td>
<td>High risk women</td>
<td>1606/462</td>
<td>64</td>
<td>30</td>
<td>2.1</td>
</tr>
<tr>
<td>Silverberg et al (2002)</td>
<td>Multi-state: HERS</td>
<td>High risk women</td>
<td>2032/551</td>
<td>63.7</td>
<td>29.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Moscicki et al (2008)</td>
<td>16 sites</td>
<td>High risk adolescents</td>
<td>133/55</td>
<td>77.4</td>
<td>54.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Abdi et al (2000)</td>
<td>Maryland</td>
<td>High risk women</td>
<td>184/84</td>
<td>69.6</td>
<td>26.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Cu-Uvin et al (1999)</td>
<td>4 sites</td>
<td>High risk women</td>
<td>840/431</td>
<td>64.3</td>
<td>27.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Massad et al (1999)</td>
<td>WHS study (6 sites)</td>
<td>High risk women</td>
<td>1517/434</td>
<td>63.2</td>
<td>30.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Palefsky et al (1999)</td>
<td>WHS study (6 sites)</td>
<td>High risk women</td>
<td>1778/500</td>
<td>63.4</td>
<td>29.8</td>
<td>2.1</td>
</tr>
<tr>
<td><strong>Other Americas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Queiroz et al (2004)</td>
<td>Brazil</td>
<td>Gynaecological clinic</td>
<td>20/35</td>
<td>100.0</td>
<td>65.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Volkow et al (2001)</td>
<td>Mexico</td>
<td>High risk women: HIV infected</td>
<td>85/44</td>
<td>67.1</td>
<td>27.3</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vs. sex workers</td>
<td>85/55</td>
<td>67.1</td>
<td>25.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Ferrera et al (1997)</td>
<td>Honduras</td>
<td>High risk women</td>
<td>23/28</td>
<td>56.5</td>
<td>17.9</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weissborn et al (2003)</td>
<td>Germany</td>
<td>Women with abnormal cervix</td>
<td>212/196</td>
<td>57.5</td>
<td>45.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Petter et al (2000)</td>
<td>Austria</td>
<td>Gynaec- and AIDS clinics</td>
<td>20/45</td>
<td>90.0</td>
<td>84.4</td>
<td>1.1</td>
</tr>
<tr>
<td>Torrisi et al (2000)</td>
<td>Italy</td>
<td>HIV+ and HIV- women</td>
<td>104/106</td>
<td>53.8</td>
<td>6.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Ubetti-Foppa et al (1998)</td>
<td>Italy</td>
<td>HIV+ and low risk controls</td>
<td>168/100</td>
<td>91</td>
<td>48</td>
<td>1.9</td>
</tr>
<tr>
<td>Cappiello et al (1997)</td>
<td>Italy</td>
<td>High risk women</td>
<td>134/98</td>
<td>40.3</td>
<td>29.6</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Africa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baay et al (2004)</td>
<td>Zimbabwe</td>
<td>Rural community women</td>
<td>61/174</td>
<td>54.0</td>
<td>27.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>68 HIV-2+</td>
<td>61.8</td>
<td>25.3</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28 HIV1&amp;2+</td>
<td>67.8</td>
<td>25.3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3633</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayaud et al (2001)</td>
<td>Tanzania</td>
<td>Antenatal clinic</td>
<td>96/511</td>
<td>34</td>
<td>34</td>
<td>1.0</td>
</tr>
<tr>
<td>Womack et al (2000)</td>
<td>Zimbabwe</td>
<td>Outpatient clinic</td>
<td>249/217</td>
<td>64.3</td>
<td>27.6</td>
<td>2.3</td>
</tr>
<tr>
<td>Marais et al (2000)</td>
<td>South Africa</td>
<td>Female sex workers</td>
<td>47/52</td>
<td>85.1</td>
<td>42.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Miotto et al (1996)</td>
<td>Malawi</td>
<td>Low and high risk women</td>
<td>129/155</td>
<td>48</td>
<td>23</td>
<td>2.1</td>
</tr>
<tr>
<td>Langley et al (1996)</td>
<td>Senegal</td>
<td>Female sex workers</td>
<td>125/556</td>
<td>56.0</td>
<td>40.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Seck et al (1994)</td>
<td>Senegal</td>
<td>High risk women</td>
<td>16 HIV-1+</td>
<td>75.0</td>
<td>20.8</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 HIV-2+</td>
<td>73.3</td>
<td>5.8</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chen et al (2005)</td>
<td>Taiwan</td>
<td>Hospital based</td>
<td>31/124</td>
<td>48.4</td>
<td>20.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Rugpao et al (1998)</td>
<td>Thailand</td>
<td>Female sex partners of HIV+ male blood donors</td>
<td>224/257</td>
<td>12.9</td>
<td>5.8</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Table 2. Prevalence of low grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) lesions in HIV-positive and -negative women

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>% LSIL HIV+ versus HIV-</th>
<th>LSIL prevalence ratio (HIV+/HIV-)</th>
<th>% HSIL HIV+ versus HIV- (HSIL prevalence ratio HIV+/HIV-)</th>
<th>HSIL prevalence ratio (HIV+/HIV-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wright et al (1994)</td>
<td>US</td>
<td>13.1 vs 3.6*</td>
<td>3.6</td>
<td>7.0 vs 0.6*</td>
<td>11.7</td>
</tr>
<tr>
<td>Six et al (1998)</td>
<td>France</td>
<td>19.0 vs 5.0</td>
<td>3.8</td>
<td>7.5 vs 2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Massad et al (1999)</td>
<td>US</td>
<td>14.9 vs 2.3</td>
<td>6.5</td>
<td>2.3 vs 1.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Maiman et al (1998)</td>
<td>US</td>
<td>15.4 vs 3.6</td>
<td>4.3</td>
<td>7.9 vs 1.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Leroy et al (1999)</td>
<td>Rwanda</td>
<td>14.6 vs 4.6</td>
<td>3.2</td>
<td>9.7 vs 1.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Motti et al (1996)</td>
<td>Malawi</td>
<td>15.0 vs 7.0*</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temmerman et al (1999)</td>
<td>Kenya</td>
<td>-</td>
<td>17.6 vs 5.2</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Mayaud et al (2001)</td>
<td>Tanzania</td>
<td>10.0 vs 6.4*</td>
<td>1.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chirenje et al (2002)</td>
<td>Zimbabwe</td>
<td>9.7 vs 1.1</td>
<td>8.8</td>
<td>3.4 vs 1.1</td>
<td>3.1</td>
</tr>
</tbody>
</table>

*histologically confirmed cervical intraepithelial neoplasia (CIN)1; †histologically confirmed CIN2-3; ‡any SIL.
Table 3. Comparison of one-year incidence of squamous intraepithelial lesions (SIL) between HIV-positive and -negative women

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>Sample size</th>
<th>Incidence SIL in HIV+</th>
<th>Incidence SIL in HIV-</th>
<th>Incidence ratio HIV+/HIV-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six et al (1998)</td>
<td>US</td>
<td>271 / 171</td>
<td>20.5%</td>
<td>4.9%</td>
<td>4.2</td>
</tr>
<tr>
<td>Massad et al (2001)</td>
<td>US</td>
<td>1639 / 452</td>
<td>8.9%</td>
<td>2.2%</td>
<td>4.0</td>
</tr>
<tr>
<td>Schuman et al (2003)</td>
<td>US</td>
<td>774 / 391</td>
<td>11.5%</td>
<td>2.6%</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Table 4. Standardized incidence ratio’s (SIR) for ICC in women with HIV or AIDS trough AIDS - cancer registry linkage studies.

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>Number of women with HIV/AIDS</th>
<th>Nr of ICC observed</th>
<th>SIR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dal Maso (2003)</td>
<td>12 cancer registries in Italy (23% of population) 1985 – 1998</td>
<td>2,663</td>
<td>18</td>
<td>21.8 (12.9 – 34.6)</td>
</tr>
<tr>
<td>Allardice (2003)</td>
<td>Scotland, UK 1980 –1996</td>
<td>2,574</td>
<td>1</td>
<td>1.7 (0.04 – 9.26)</td>
</tr>
<tr>
<td>Clifford (2005)</td>
<td>9 cancer registries in Switzerland (56% population) 1985 – 2002</td>
<td>2,045</td>
<td>6</td>
<td>8.0 (2.9 – 17.4)</td>
</tr>
<tr>
<td>Newnham 2005</td>
<td>South-East England, UK 1985 – 2001</td>
<td>7,110</td>
<td>3</td>
<td>1.0 (0.2 – 2.9)</td>
</tr>
<tr>
<td>Engels 2006</td>
<td>11 areas in the U.S. (same as Frisch (2001)) 1996 – 2002</td>
<td>27,282</td>
<td>30</td>
<td>5.3 (3.6 – 7.6)*</td>
</tr>
<tr>
<td>Mbulaiteye 2006</td>
<td>Kyadondo County, Uganda 1989 – 2002</td>
<td>8,423</td>
<td>137</td>
<td>2.7 (1.8 – 4.0)</td>
</tr>
</tbody>
</table>

* period of SIR assessment is during 4 – 27 months post-AIDS
2. OBJECTIVES AND METHODOLOGY

2.1. General Objective

The general objective of this work is to describe the interaction between HPV and HIV, the epidemiology of human papillomavirus in Kenyan populations and the prevalence of HPV types and the impact of HIV in invasive cervical cancers in Kenya.

2.2. Specific Objectives

1. To describe the prevalence of HPV types among women in Nairobi and Mombasa. As population-based HPV prevalence data are scanty for Africa in general, we wanted to assess this information in family planning populations, as an approximation of the general population.

2. To describe the prevalence of HPV types in invasive cervical cancer in Nairobi and the impact of HIV infection on HPV type distribution in these women. This was done at the national referral hospital, which attracts patients with advanced pathology from all over the country.

3. To compare test characteristics of several alternative screening methods for detection of cervical pre-cancer and cancer in poor-resource regions applied to a family planning clinic setting in Nairobi.
2.3. Study sites and study development

Kenya was chosen to perform these studies, because the burden of cervical cancer in East Africa is one of the highest in the world. The Ghent University also had a long standing collaboration with the University of Nairobi on topics of sexually transmitted diseases including HIV and cervical cancer.

Background demographic statistics Kenya (WHO World Health Statistics 2007, Figures are for 2005 unless indicated.)

- Total population: 34,256,000
- Gross national income per capita (PPP international $): 1,170
- Life expectancy at birth m/f (years): 51/51
- Healthy life expectancy at birth m/f (years, 2002): 44/45
- Probability of dying under five (per 1,000 live births): 120
- Probability of dying between 15 and 60 years m/f (per 1,000 population): 464/483
- Total expenditure on health per capita (Intl $, 2004): 86
- Total expenditure on health as % of GDP (2004): 4.1
Several studies were performed to obtain these data:

*Project Reproductive Health (PRH):*
This study was a collaboration between the Ghent University (GU), Antwerp University, the University of Nairobi (UoN) and the Family Planning Association of Kenya (FPAK) and was sponsored by the Flemish Interuniversity Council (VLIR). Five hundred sixteen women seeking family planning services were screened for sexually transmitted infections, cervical cancer (and precursors) and HPV between 1998 and 2000.

The study was carried out at Ribeiro clinic, one of the FPAK family planning clinics in the Nairobi city centre. Nairobi counts around 2.3 million inhabitants, of whom an estimated 1 million live in slum settlements. The FPAK was greatly subsidised by foreign development aid agencies and could therefore offer high quality family planning and reproductive health services for a modest service fee. The clinic provided one examination room for the study. One study nurse and one medical doctor were employed and trained for the study. The clinic staff assisted in awareness creation and recruitment of the study participants. Monthly clinic feedback meetings were held to discuss study procedures and findings.

The study was approved by the Kenyatta National Hospital Ethical and Research Committee and all participants gave a written informed consent.

*Cervical cancer in Kenya, its relation to HIV infection:*
This was a collaborative study between the Ghent University (GU) and the University of Nairobi (UoN), sponsored by the Flemish Interuniversity Council (VLIR). Two hundred and five women with ICC were screened for HIV and HPV and followed up for one year post treatment between 2000 and 2003.

Every year, the university teaching hospital, Kenyatta National Hospital (KNH), admits around 250 women with ICC. Patients accessing the hospital are self-referral from Nairobi city, and referred patients from the whole of Kenya. KNH is the only public facility offering radiotherapy treatment in Kenya. The study recruited cervical cancer patients seeking radiotherapy treatment at KNH. Study participants were examined in a dedicated study room by a gynaecologist and trained study nurse.
The study was approved by the Kenyatta National Hospital Ethical and Research Committee and all participants gave a written informed consent.

*Mombasa cervical cancer screening project (MCCSP):*
A Collaboration between the Ghent University (GU), Min of Health Kenya, the University of Nairobi (UoN), Makerere University (Uganda) and Institute for Research and Cure (Milan, Italy), sponsored by the European Commission (INCO-Dev). Three thousand nine hundred twenty-seven women from the population were screened at 9 primary health care centres (PHC) for cervical cancer (and precursors) and 663 for HPV between 2002 and 2005.

The PHC were run by either the Mombasa City Council or directly by the Provincial Medical Officer. They all refer to the Coast Provincial General Hospital (CPGH), which is the second largest hospital in Kenya. It is a teaching and referral hospital whose service area includes the seven districts in the Coast Province. The primary service area (Mombasa District) has a population of over 600,000. The predominant activity has been service delivery as for all government hospitals in Kenya. For the past +-15 years, it has also been involved in various research activities with the University of Nairobi, the University of Washington (US), the International Centre for Reproductive Health (Ghent University) and others.

Mombasa is a port town with a high incidence of sexually transmitted infections, HIV and cervical cancer. There was no major cytology lab in Mombasa. The study set up a cytology lab at the CPGH. For this purpose we recruited a graduate from the VLIR ‘Masters Training in Clinical Cytology’ course (University of Nairobi, 1995 – 2002), for which I was the project advisor. Within the MCCSP, there was also a nested study to assess the test characteristics of Pap smear in the detection of bacterial vaginosis [Karani et al., 2007].

The study was approved by the Kenyatta National Hospital Ethical and Research Committee and all participants gave a written informed consent.

More detailed information on the methodologies of the studies can be found in the “methods and materials” sections of the papers in the following results section.
2.4. Dissemination of results

Data dissemination was done through presentations at yearly seminars at the University of Nairobi (1998 – 2004). These seminars were attended by local and international scientists, as well as representatives of the local health authorities. The data have also been presented at several international scientific conferences.

2.4.1. Presentations at Scientific conferences

Oral Presentations:


*Poster Presentations:*


De Vuyst H., Lillo F., Karani A., Lodini S., Temmerman M. Age-specific HPV type
distribution among women in Mombasa, Kenya. 23rd International Papillomavirus
Conference 2006, Prague, Czech Republic, September 01-07, 2006.

2.4.2. Publications in Peer reviewed journals


* De Vuyst H, Steyaert S, Van Renterghem L, Claeys P, Muchiri L, Sitati S,
of Human Papillomavirus in a Family Planning Population in Nairobi, Kenya. *Sex
Transm Dis* 30:137-142

Integration of cervical screening in family planning clinics. *Int J Gynaecol Obstet*
81:103-108

* Gichangi P, Bwayo J, Estambale B, De Vuyst H, Ojwang S, Rogo K, Abwao H,
women. *AIDS* 17: 1-6

Performance of the acetic acid test when used in field conditions as a screening test
for cervical cancer. *Trop Med Int Health* 8: 704-709

* De Vuyst H, Claeys P, Njiru S, Muchiri L, Steyaert S, De Sutter P, Van Marck E,
Bwayo J, Temmerman M (2005) Comparison of pap smear, visual inspection with
acetic acid, human papillomavirus DNA-PCR testing and cervicography. *Int J
Gynaecol Obstet* 89: 120-126

1 * Papers are included in the results section, others are provided in annex.


Paper in development to be submitted to the British J of Cancer:
3. RESULTS


Distribution of Human Papillomavirus in a Family Planning Population in Nairobi, Kenya

The authors thank the Family Planning Association of Kenya for its continued support of the study by allowing the use of its premises and highly motivated staff, without whom the study would not have been possible, and Miss Nadia El Mahi, who secured the communication lines between Kenya and Belgium.

Supported by a grant from VLIR (Flemish Interuniversity Council).

Correspondence: Marleen Temmerman, MD, PhD, ICHR, UZ IJPE, De Pintelaan 185, 9000 Gent, Belgium. E-mail: marleen.temmerman@rug.ac.be

Received March 5, 2002, revised July 12, 2002, and accepted July 15, 2002.

Background: In sub-Saharan Africa, cervical cancer is the leading cancer among women. The causative role of different human papillomavirus (HPV) types in cervical cancer is established, but the distribution of HPV types within this region is largely unknown.

Goal: The goal was to study the distribution of HPV among family planning clinic attendees in Nairobi, Kenya.

Study Design: This was a cross-sectional study of persons attending a family planning center in Nairobi, Kenya.

Results: HPV data of 429 women were analyzed; 7.0% had low-grade intraepithelial lesions, 6.8% had high-grade intraepithelial lesions, and 6.2% had invasive cancer. One hundred ninety samples (44.3%) were HPV-positive (28.4% were positive for multiple types). The most common HPV types were HPV 52 (47.9% of positive samples), HPV 16 (47.7%), HPV 35 (11.6%), and HPV 66 (9.0%). The risk of high-grade squamous intraepithelial lesions (HSIL) was 88.5 times higher (95% CI, 8.5–1.4 × 10^6) in HPV 16-positive women than in HPV-negative women. Relative risks were 54.3 (95% CI, 4.0–1.4 × 10^4) for HPV 35, 40.2 (95% CI, 3.6–9.5 × 10^4) for HPV 52, and 21.7 (95% CI, 6.0–1.9 × 10^5) for HPV 18. The prevalence of HSIL was not increased in association with HIV-positivity, yet HIV-1 was significantly associated with high-risk HPV types (P < 0.00001).

Conclusion: The pattern of HPV distribution in this population was different from that in other regions in the world, which has important consequences for HPV vaccine development.
Kenya, were invited to participate in a cross-sectional study to evaluate different cervical cancer screening techniques. The collected data included sociodemographic, medical, and reproductive health variables and the findings of a Papanicolaou smear, visual inspection with acetic acid (VIA) for the detection of cervical lesions, and colposcopy, performed on all women. HPV testing was done in a random subset of the study sample. Serum samples were obtained for HIV testing after pretest counseling. Written informed consent was obtained from all participants.

**Biologic Specimens**

Exfoliative cervical cells were obtained with a Cervex brush (Rovers Medical Devices, Oss, The Netherlands). One smear was made on a glass slide for staining according to the Papanicolaou (Pap) method. The brush was then submerged and stirred in 10 ml phosphate buffered saline, and the specimen was frozen at −20°C. Samples were shipped to the Laboratory of Virology, Ghent University Hospital, Belgium, for HPV DNA extraction, detection, and genotyping. Pap smears and cervical biopsies were processed and analyzed at the Department of Human Pathology, University of Nairobi, Kenya, according to the Bethesda classification. Quality control was done on a subsample of the Pap smears, and all histology specimens were examined at the Department of Pathology, University of Antwerp, Belgium. For final data analysis, the histologic findings from Antwerp University were used. Venous blood samples were tested for HIV-1 and HIV-2 with use of the ELISA Detect HIV 1/2 (Immunosystems, Montreal, Quebec, Canada) and the Recombigen HIV 1/2 (Trinity Biotech, Galway, Ireland). Scientists performing the biologic assays were masked to the clinical diagnoses.

**HPV DNA Extraction, Detection, and Typing**

HPV DNA was isolated by incubating the samples with proteinase K, as described previously. The proteinase was heat-inactivated. Broad-spectrum HPV DNA amplification was performed with the short PCR fragment (SPF10) primer set, as described previously. The SPF primers generate a 65-bp fragment from the L1 region of the HPV genome. The PCR products were analyzed by HPV DNA enzyme immunoassay (DEIA), a microtiter plate-based hybridization assay, as described previously. Samples identified as HPV-positive were genotyped with the INNO-LiPA HPV prototype reaction assay. In this assay, the HPV PCR product is reverse-hybridized to genotype-specific probes immobilized as parallel lines on a nitrocellulose strip. Twenty-five HPV genotypes (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, and 74) can be identified simultaneously in a single hybridization step. HPV amplimers that do not hybridize to any probe are assigned HPV genotype X (uncharacterized HPV type). The presence and adequacy of amplification of DNA were checked in a second reaction with HLA-DQ primers GH26 and GH27. This experiment was also controlled with separate positive and negative PCR controls.

**Statistical Analysis**

Relative risks (RRs) with 95% confidence intervals (CIs) were used to measure associations in univariate analysis. They were calculated by exact methods, except when all expected cell counts exceeded 5. In the calculation of RRs for particular HPV types, regardless of single or mixed infection. RRs adjusted for age were obtained in log-linear risk models. In the second and third tables and the second figure, we classify HPV-positive samples as containing HR types, exclusively LR types, or type X. The analysis was performed with SPSS version 10.0.5 (SPSS, Chicago, IL). Exact confidence intervals were calculated with StatXact-5 (Cytel, Cambridge, MA).

**Results**

**Characteristics of the Population**

We recruited a total of 816 women. Six hundred ninety-three attended the clinic for regular family planning services; 123 were transferred because of clinical symptoms or a recent positive Papanicolaou smear. We excluded the latter group for the current report. Of the former group, 548 presented for the second visit, at which the reference test was performed. HPV results are available for 445 women of this group. Not all patients had an HPV sample taken, because this depended on the availability of the PBS sampling medium at the clinic.

Four HPV tests had a borderline (unequivocal) result, and 12 results were indeterminate (4 negative HLA-DQ results, 5 possible contaminations due to a broken vial, 1 case of duplicate numbering, 1 vial with insufficient material, and 1 aspecific INNO-LiPa result). These cases were also excluded from the analysis.

Finally, the data of 429 participants were analyzed and are presented in this study report. The mean age (standard deviation [SD]) was 35.2 (6.5) years. The average (SD) reported age at first sexual intercourse was 19.5 (3.3) years. The proportion of women with 4 or more lifetime sexual partners was 23.4%; 43.4% of women reported having had a Pap smear done in the past 5 years and 28.9% in the past 2 years. A history of symptoms compatible with a genital infection was reported by 17.2% of the study sample.

The findings of the cervical dysplasia reference test were as follows: 30 cases (7.0%) of cervical intraepithelial neoplasia grade 1 (CIN 1) or LSIL, 9 (2.1%) of CIN 2, 20 (4.7%) of CIN 3 (29 cases [6.8%] of HSIL), and 1 of invasive cancer (0.23%). Figure 1 shows the age-specific distribution of LSIL and HSIL in the population. The prevalence of LSIL is highest in the youngest women (14.5% in the age group of 25–29 years) and decreases with age. The association between age and LSIL was strongly significant (P = 0.0040). The apparent rise of the curve after the age of 44 years could not be confirmed in a logistic regression model (P = 0.16). The prevalence of HSIL reaches a peak in the age group of 35 to 39 years (8.8%). We could not show a significant correlation between age and HSIL (P = 0.238).
Prevalence of HPV Types

One hundred ninety samples (44.3%) were HPV-DNA-positive by SPF10 PCR. The prevalence of HPV types is described in Table 1. A total of 23 different HPV types was detected. Of the positive samples, 136 (71.6%) were positive for a single HPV type, 54 (28.4%) were samples containing more than one HPV type (mixed: 17.3%, two types; 8.5%, three types, 1.5%, four types; and 1.1%, five types). The prevalence of HPV was highest in the younger age groups (57.9% in the age group of 25–29 years) and decreased with age (25% in the age group of 50–54 years) (Figure 1). The most common HPV types were HR types HPV 52 (17.9% of positive samples), HPV 16 (14.7%), HPV 35 (11.6%), and HPV 66 (9.0%). An uncharacterized type (type X) was found in 15.8% of the HPV-positive samples. Overall, 272 occurrences of individual HPV types were observed (single/mixed infections). Of those, 188 (69.1%) were HR types, 54 (19.9%) were LR types, and 30 (11.0%) were type X. HPV 52 was observed as a single HPV type in 14 cases and mixed with other HPV types in 20 cases, yielding a ratio (single/mixed) of 14/20. Corresponding ratios are 18/10 for HPV 16, 9/11 for HPV 35, 6/11 for HPV 66, and 5/6 for HPV 18.

Figure 2 shows the distribution of HPV by oncogenicity in the total group, the normal group, the LSIL group, and the HSIL group. Of the total study sample, 55.7% was HPV-negative, which was the case for 61.1% of women with normal cytological findings, 40.0% of the LSIL cases, and 3.3% (1 case) of the HSIL group. In all groups, the HR HPV types were the most prevalent ones. In the group with a normal cervix, HR HPV types were found in 61.5% of the positive HPV samples; in the LSIL and HSIL group, the percentages were 88.9% and 96.4%, respectively (Table 2).

Table 2 shows the frequency of the different HPV types within the cytopologically normal, LSIL, and HSIL groups and the RRs of HSIL in different types of HPV-positive versus HPV-negative cases. The three most frequent HPV types in the HSIL group are HPV 16 (35.7% of positive samples), HPV 52 (25.0%), and HPV 35 (17.9%). Within the group without invasive cancer, the risk of...
HSIL was 35.4 times and 49.3 times higher for HPV-positive and HR HPV-positive women, respectively, than for HPV-negative women. The strongest associations with HSIL were found for HPV 16 (RR = 88.5), HPV 35 (RR = 54.3), HPV 52 (RR = 49.2), and HPV 18 (RR = 21.7). Except for the last one, these RRs are all significantly different from 1 at the 5% level. Unadjusted RRs approximate well the age-adjusted RRs for the individual subtypes (not shown in table). Only weak associations were found for LSIL and HPV infection (RR for any HPV = 2.2; 95% CI, 1.1–4.5; RR for HR HPV = 3.1; 95% CI = 1.2–9.0). No significant associations were found between LSIL and individual HPV types, except for HPV 35 (RR = 8.1; 95% CI = 2.1–28.3).

Table 3 correlates the HPV and SIL results with HIV-1 serostatus. Of the 409 cases with an HIV test result, 362 were HIV-negative (88.5%) and 47 were HIV-1-positive (11.5%). Nobody was HIV-2-positive. A HR HPV type was observed in 74.5% of HIV-1-positive women and 24.9% of HIV-negative women (age adjusted RR = 2.82; 95% CI = 2.2–3.6). Only two HSIL cases (4.3%) were found in the HIV-1-positive group, compared with 26 (7.2%) in HIV-negative group. Among HIV-negative women, the risk of HSIL was 60.0 times higher (95% CI = 8.2–440.2) for HR

**Table 2. Relative Risk for SIL in Association With Type-Specific HPV**

<table>
<thead>
<tr>
<th>HPV Status</th>
<th>n</th>
<th>HPV-Positive (%)</th>
<th>n</th>
<th>HPV-Positive (%)</th>
<th>RR</th>
<th>95% CI</th>
<th>RR a</th>
<th>95% CI a</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV negative</td>
<td>226</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV positive</td>
<td>143</td>
<td>100.0</td>
<td>18</td>
<td>28</td>
<td>35.4</td>
<td>4.9–257.9</td>
<td>34.8</td>
<td>5.07–239.4</td>
</tr>
<tr>
<td>HPV LR</td>
<td>27</td>
<td>18.9</td>
<td>1</td>
<td>5.6</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV HR</td>
<td>88</td>
<td>61.5</td>
<td>16</td>
<td>88.9</td>
<td>27</td>
<td>96.4</td>
<td>49.3</td>
<td>6.8–358.4</td>
</tr>
<tr>
<td>HPV X</td>
<td>28</td>
<td>19.6</td>
<td>1</td>
<td>5.6</td>
<td>3.6</td>
<td>8.0</td>
<td>0.0–5.6×10⁸</td>
<td>7.81</td>
</tr>
<tr>
<td>HPV LR</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>9</td>
<td>6.3</td>
<td>1</td>
<td>5.6</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>10</td>
<td>7.0</td>
<td>3</td>
<td>16.7</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>8</td>
<td>5.6</td>
<td>2</td>
<td>11.1</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>6</td>
<td>4.2</td>
<td>1</td>
<td>5.6</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>7</td>
<td>4.9</td>
<td>1</td>
<td>5.6</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV HR</td>
<td>16</td>
<td>13</td>
<td>9.1</td>
<td>4</td>
<td>22.2</td>
<td>10</td>
<td>35.7</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>8</td>
<td>5.6</td>
<td>2</td>
<td>11.1</td>
<td>1</td>
<td>3.6</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>12</td>
<td>8.4</td>
<td>1</td>
<td>5.6</td>
<td>2</td>
<td>7.1</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>7</td>
<td>4.9</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3.6</td>
<td>29.9</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>10</td>
<td>7.0</td>
<td>7</td>
<td>38.9</td>
<td>5</td>
<td>17.9</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>5</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>6</td>
<td>4.2</td>
<td>1</td>
<td>5.6</td>
<td>2</td>
<td>7.1</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>4</td>
<td>2.8</td>
<td>1</td>
<td>5.6</td>
<td>3</td>
<td>10.7</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>23</td>
<td>16.1</td>
<td>4</td>
<td>22.2</td>
<td>7</td>
<td>25.0</td>
<td>49.2</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>5</td>
<td>3.5</td>
<td>1</td>
<td>5.6</td>
<td>1</td>
<td>3.6</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>10</td>
<td>7.0</td>
<td>2</td>
<td>11.1</td>
<td>2</td>
<td>7.1</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>1</td>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>13</td>
<td>9.1</td>
<td>1</td>
<td>5.6</td>
<td>3</td>
<td>10.7</td>
<td>42.2</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>6</td>
<td>4.2</td>
<td>1</td>
<td>5.6</td>
<td>2</td>
<td>7.1</td>
<td>53.1</td>
</tr>
</tbody>
</table>

*Normal reference test.
†Relative risk, adjusted for age. Not calculated for type-specific RR because of poor small-sample performance.
§Approximate (asymptotic) 95% CI.
| HPV = human papillomavirus; HR = high risk; HSIL = high-grade SIL; LR = low risk; LSIL = low-grade SIL; RR = relative risk; SIL = squamous epithelial lesions.
HPV IN A NAIROBI FAMILY PLANNING POPULATION

Vol. 30 • No. 2

TABLE 3. Association Between HIV-1 and HPV Prevalence

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-1-Negative (n = 362)</th>
<th>HIV-1-Positive (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-1-Positive</td>
<td>HSIL (%)</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>n%</td>
<td>n%</td>
</tr>
<tr>
<td>HPV LR</td>
<td>222</td>
<td>61.3%</td>
</tr>
<tr>
<td>HPV HR</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV X</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>222</td>
<td>61.3%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>6.9%</td>
</tr>
<tr>
<td>HPV-negative</td>
<td>25</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

*One woman with HSIL had no HIV-1 test result, so the total number of HSIL cases is 28 in this group.

HPV–positive than HPV-negative women after adjustment for age. No significant association between HSIL and HR HPV was found for HIV-1-positive women.

Discussion

This study report is one of the first to describe the prevalence and distribution of HPV types within an East-African population. We describe a population of family planning clinic attendees, with a mean age of 35.2 years, in an urban setting (Nairobi, Kenya). They scored low for the classic risk factors for cervical dysplasia (sexual and Pap smear history). However, in our study sample, 44.3% were infected with HPV and 30.8% with an HR type. This is one of the highest prevalences in unselected populations reported to date. Previous studies reported HPV prevalences of 39% in Honduras (21% of positives were infected with HR HPV types); 16% in Costa Rica (7.6% HR HPV types in the control population); 21% in Morocco; 17% in Brazil and Colombia; 16% in Thailand; 9% in the Philippines; and 6% in Spain. In East Africa, HPV types 52, 16, and 35 were most prevalent. HPV 16 was not the sole predominant type in our population, in contrast to most other studies, conducted in both random populations and selected cervical cancer or precancer populations.32, 33, 34, 35, 36, 37

In our study, HPV 52 was more prevalent than HPV 16. In general, however, HPV 52 is among the less prevalent HPV types, occurring at rates such as 0% in HPV-positive women in Thailand (1998), 6% in the Philippines (1998), and 0% in Paraguay.38 To our knowledge, this HPV type has only once been reported to be the second most prevalent HPV type in an unselected population (Thailand, 2000).3 We describe these comparisons with caution, as we realize that these studies have used different HPV primers that can have different sensitivities for different HPV types. The fact that only one woman in 29 with HSIL was not infected with HPV adds evidence of the role of HPV in cervical carcinogenesis. We found, not surprisingly, that the association between an HR HPV type and HSIL was strong. The greatest association was seen with HPV 16 (RR of HSIL = 88.5), HPV 35 (RR = 54.3), and HPV 52 (RR = 49.2).

These findings are in accord with results of a study in Honduras,8 which showed significant odds ratios for the association between cervical intraepithelial neoplasia (CIN) grade III and infection with HPV 16–related and HPV 18–related viruses (ORs of 48 and 24, respectively). In our population, HPV 52 was not only highly prevalent but also highly oncogenic. There was no evidence in the data of a difference in oncogenicity of HPV 52 as a single or multiple infection (P = 0.60). Not a single HSIL case was associated with a low-risk HPV type or was part of a mixed-type sample. Only one case of HSIL was associated with an uncharacterized HPV type, yielding a low and insignificant association.

This finding suggests that these types pose a low risk for HSIL and cervical cancer. Not much evidence is available on the carcinogenic potential of HPV 66. It was described as an HR HPV type on the basis of phylogenetic analysis11 and recently was noted in one case of cervical cancer.10 In our study, however, we found HPV 66 in 9% of the HPV-positive samples, and the risk of HSIL was estimated to be 42 times higher in HPV-66-positive women than in HPV-negative women. This illustrates the relative importance of this type in our population.

The association between LSIL and HPV was weak, which is in agreement with findings of the Honduran study. In the Costa Rican study, 30% of the LSIL cases were HPV-negative.10 This was of concern to the investigators because they postulated that all LSILs are the result of an active HPV infection and therefore are HPV-positive. In our study, we noted a similarly high proportion (40%) of HPV-negative LSIL cases. Whereas LSIL was seen in the younger age groups, HSIL was most prevalent in older women (aged 30 to 39 years). This is consistent with previous findings.33, 34

We conducted this research in a population with high HIV-1 prevalence (11.5%). The positive associations between HIV-1 and HPV, HIV-1 and SIL, and HPV and SIL have been established in most studies.35–38 However, contradictory findings were reported from Nairobi following a study among commercial sex workers in which no association was seen between SIL and HIV-1 and only a weak association was seen between HIV-1 and HPV.39, 40 We found a significantly positive association between HIV-1 and HR HPV infection, but we did not find a higher prevalence of HSIL among HIV-1-positive women. However, the number of HIV-1-positive HSIL lesions was very small, making it difficult to establish any possible association.

We recognize that our study has certain limitations. Although we did not conduct a methodically designed population-based study, we think that women attending the considered family planning clinic are representative of those in other urban settings in East Africa. Hence, the prevalences found reflect an estimate of HPV prevalences and distribution of specific types within various East-African populations. In summary, our study demonstrates that HPV is highly prevalent in this East-African population with a high prevalence of cervical precancer. The most frequent types were HR HPV 16, 18, 31, 35, 52, 53, 58, and 66. The distribution of HPV types varies substantially by region,7 and for the first time, the importance of HR HPV types 52 and 66 was demonstrated.

Although it could be sufficient to develop effective HPV vaccines against only a few of the predominant HPV types in a certain region, further epidemiologic studies are warranted to identify these HPV types carefully.37

References


3.2. Human papillomavirus infection in Mombasa, Kenya: a population-based study among family planning attenders.


This paper is in development phase to be submitted to the British Journal of Cancer.

References from this section are listed in Section 9.

MATERIALS AND METHODS

Study subjects
This study was nested within an operational cervical cancer screening study, assessing the feasibility of introducing cervical cancer screening at the level of Primary Healthcare Centre (PHC) of Mombasa District, Kenya, a semi-urban area of 600,000 inhabitants. The population was heterogeneously composed of local coastal people and inland people who migrated to Mombasa in order to find work in the port-associated economy. Sex for money or favours is common in this community. Screening through pap smear and visual inspection with acetic acid (VIA) was introduced in 9 PHC and women were referred to the Coast Provincial General Hospital (CPGH) (secondary level) for colposcopy, biopsy taking and treatment if needed. Women older than 15 years were recruited from 5 different suburban areas around the town, either at the occasion of spontaneous visits for family planning or mother-child health care services at the PHC, either through a referral by community health workers (CHW) who created awareness of the cervical screening programme in the areas around the PHC. The study enrolled women between March 2002 and May 2005.

Within this screening study, we aimed to enroll approximately 100 women in each 5-year age group between 15 – 19 and 55 – 59 years for the HPV study. Women were consecutively enrolled until completion of the respective 5-year age groups.
After explanation of the study goals and procedures, participating women were interviewed about socio-demographics and sexual history by trained study nurses, after which the nurses performed a pelvic examination, filled in the clinical report form (CRF) and study samples were taken.

All participants signed informed consent forms, according to the recommendations of the Kenyatta National Hospital Ethical Review Board, which approved the study.

**Gynaecological examination and cervical specimen collection**

A total of 663 women were enrolled in the HPV study and after a structured interview was taken, all underwent a pelvic examination. Signs and symptoms of the examination were noted in the CRF. Cervical exfoliated cells were collected by rotating a cervex brush (Rovers Medical Devices, Oss, The Netherlands) five times at the cervical os, collecting endocervical as well as ectocervical cells from the transformation zone. The brush was smeared on a glass slide for pap smear, which was immediately immersed in 95% ethanol fixative, after which the tip of the brush was kept in Standard Transport Medium for HPV testing (Digene Corp. Bethville). Then the nurse performed visual inspection with acetic acid (VIA), as described before [De Vuyst et al., 2005]. In case a lesion was seen on VIA or other any other clinical findings suggesting gynaecological follow-up, the woman was immediately referred for colposcopy at the CPGH. Women visited the PHC centre again, three weeks after pap smear taking to receive the cytological results. In case any lesion of atypical squamous cells of undetermined significance (ASCUS) or worse was detected, the woman was also referred for colposcopy to the CPGH. Specimen were transported to the CPGH, where the HPV specimen was frozen at –20 Celsius. Colposcopy was performed by trained colposcopists. Colposcopic impression was noted in the CRF and in case a lesion was seen, a biopsy was taken. In case of an abnormal screening test and no visible colposcopic lesions, an endocervical curettage (ECC) was performed.

**Cytology and histology**

The pap smear slides were processed at the histology lab at the CPGH, where the slides were processed by trained cytotechnologists and read by two of us: Mr. A. Karani (MSc clinical cytology) under the supervision of Dr. K. Mandaliya (Provincial Pathologist). Cytology was reported according to the Bethesda classification.
The Bethesda system for reporting cervical/vaginal cytologic diagnoses: definitions, criteria, and explanatory notes for terminology and specimen adequacy. New York, N.Y.: Springer-Verlag, 1994). The biopsies and ECC’s were processed at the CPGH histology lab and read by the same pathologist. All abnormal smears, as well as 10% of negatives and all biopsies were reviewed for quality control by a teaching clinical pathologist at the Department of Human Pathology, University of Nairobi (Dr. L. Muchiri). In the present study, cervical abnormalities were defined as histological cervical intra-epithelial neoplasia grade 1 or worse (CIN1+), or cytological ASCUS or worse in case colposcopy/biopsy was missing.

**HPV detection** (HPV detection methodology will be described by Dr. Flavia Lillo)

Human papillomavirus testing was performed on exfoliated cervical cells in the Laboratory of Virology, AIDS Center "San Luigi", IRCCS Hospital San Raffaele, Milan, Italy. Basically, cervical scrapes were tested for HPV-DNA using either SPF10 – LiPA (DDL-Innogenetics) or MY09-11/GP5+6+ - LA (Roche).

**RESULTS**

Of 663 women who were enrolled in the HPV study, 19 had B-globine-negative HPV samples and another 26 women had unsatisfactory cytology results. Of the remaining 618 women, 63 biopsies were performed, of which 11 CIN1, 8 CIN2, 11 CIN3, 3 invasive squamous cell carcinoma (ICC). Another 25 women had abnormal cytological results without colposcopy/biopsy examinations of which 10 ASCUS, 11 LSIL, 3 HSIL and one suggestive of ICC. Overall, 58 (9.4%) of women had cervical abnormalities.

The prevalence of any HPV infection was 44.7% (58.6% and 43.2% among women with and without cervical abnormalities, respectively)(Table 1). In total, 23.1% of women had single-type and 20.0% had multiple-type infections. In all, 29 individual types of HPV were identified.

High-risk HPV types were more frequent (34.6% of all women) than low-risk types (21.4%). The most common types in either single- or multiple-type infections were HPV58 (9.7%), HPV16 (7.1%), HPV53 (6.5%) and HPV52 (5.5%). HPV type
distribution did not vary considerably between women with abnormal and normal cervices, except for HPV16 (12.1% and 6.6%, respectively) and HPV45 (5.2% and 1.6%, respectively). High-risk HPV types were found in 46.6% of women with abnormal, compared to 33.4% in women with normal cervix.

Figure 1 and Table 2 show the prevalence of HPV (any type, High- and low-risk types, separately) by age group. HPV prevalence was high among all age groups with a borderline significant increase in women aged 25 – 34 years, compared to those younger than 25 years (OR 1.6, 95% CI (1.0 – 2.5)).

Table 2 shows the relationship between HPV positivity and various characteristics after adjustment for age and lifetime number of sexual partners. No significant association was found between HPV positivity and age at sexual debut, lifetime number of sexual partners, number of partners during the last 12 months and husband’s extramarital sexual relationships. Other characteristics, like educational level, commercial sex work, polygamy, oral contraceptive use, smoking, use of open wood fires for cooking or history of malaria are currently under analysis.

DISCUSSION

This study is one of the largest to describe age-specific HPV prevalence in an East-African population. Compared to other population-based surveys, we found one of the highest HPV prevalences (44.7%), a high proportion of multiple-type infections and no predominant prevalence of HPV16.

Studies using PCR-based assays already demonstrated high HPV prevalence among populations in sub-Saharan Africa: 40% in rural Mozambique [Castellsague et al., 2001], 31% in Harare, Zimbabwe [Gravitt et al., 2002], 31% in women older than 35 years in Senegal [Xi et al., 2003], 44% in Nairobi, Kenya [De Vuyst et al., 2003], 34% in rural Zimbabwe [Baay et al., 2004] and 26% in Ibadan, Nigeria [Thomas et al., 2004]. A lower prevalence of 13% was found in rural Gambia, West Africa [Wall et al., 2005]. As shown before in sub-Saharan Africa, we also found a high proportion of multiple-type infections [Castellsague et al., 2001;De Vuyst et al., 2003;Gravitt et al., 2002;Thomas et al., 2004].

Age-specific curves of HPV prevalence have been shown to vary across populations worldwide [Franceschi et al., 2006]. The earliest reports from US and Northern
Europe, where a peak of HPV prevalence was seen before the age of 25 – 35, was confirmed for other higher income areas in other regions included in the IARC HPV Prevalence Surveys (IHPS), notably Argentina, Korea, Lampang in Thailand and Ho Chi Minh in Vietnam. The HPV prevalence stayed high across all ages in our study, a finding that was found in other population-based studies in poor regions with high rates of cervical cancer incidence, notably in Asia (Shanxi, China [Dai et al., 2006] and Dindigul, India [Franceschi et al., 2005]) and for sub-Saharan Africa (Nigeria [Thomas et al., 2004], South Africa [Kuhn et al., 2000], The Gambia [Wall et al., 2005] and Senegal [Xi et al., 2003]). Different age-specific patterns have been found within sub-Saharan Africa, some also declining with age in Zimbabwe [Baay et al., 2004;Womack et al., 2000], Uganda [Serwadda et al., 1999], Nairobi, Kenya [De Vuyst et al., 2003] and Mozambique [Castellsague et al., 2001].

Clifford et al (2005a) demonstrated earlier in pooled IHPS data that HPV16 was underrepresented in HPV positive women with normal cervix in Nigeria, compared to other regions in the world. Other HR HPV types were more frequent, most notably HPV35. We do not report a high prevalence of HPV35 in our study group (6.5% of HPV-positive women). HPV16 was high (16.0% of HPV-positive women), however not predominant, as other HPV types were found with similar prevalence: 21.7% HPV58, 14.5% HPV53 and 12.3% HPV52.

We did not perform HIV testing in the study group. However, we know from Kenyan sentinel data that the HIV prevalence in women in Mombasa for 2004 can be estimated at 14.3% [National AIDS Control Council, 2005]. The relatively high HIV prevalence in this group might partly explain the high prevalence of HPV infection, high proportion of multiple-type infections [Clifford et al., 2006b], but also the high HPV prevalence across all age groups, as has been described in HIV-positive women from different regions [Mayaud et al., 2001;Palefsky et al., 1999].

The difference in HPV prevalence found between women with normal cervix and abnormal cervix was small (Table 1). To our knowledge, this is the smallest difference seen to date in a population based survey. This might be explained by the overall high background HPV prevalence in the population, and the fact that over half of the abnormal cervixes were due to CIN1 – ASCUS-LSIL lesions (32/58).

No associations were found between HPV positivity and a number of classical sexual behaviour risk factors. Interestingly, a borderline significant association was found between HPV positivity and the presence of husband’s extramarital relationships,
however, this association disappeared after adjustment for the women’s own number of sexual partners. More detailed analysis is ongoing.

Our study has strengths and limitations. Among the strengths is the age-stratified sampling from different sites across the district area. This offered adequate power to the study to describe age-specific HPV prevalence in the population. A weakness is the fact that most women were selected opportunistically, as they attended the family planning clinics for FP services. Another weakness is the fact that two different HPV detection tests were used during the study period, due to budgetary reasons. We are confident however that results for both tests are comparable, as shown before on cervical scrapes in a comparison study [van Doorn et al., 2002]. The slight preference of both tests for specific HPV types could be attenuated by the mixed use of the tests.

In conclusion, our study shows one of the highest population HPV prevalences described to date with sustained high prevalence over all age groups with a lack of predominance of HPV16, as seen in pooled worldwide HPV distributions [Clifford et al., 2005a].

<table>
<thead>
<tr>
<th>Cervical abnormalities</th>
<th>Normal (N=560)</th>
<th>Abnormal (N=58)</th>
<th>Total (N=618)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Multiple</td>
<td>Total(%)</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>124</td>
<td>111</td>
<td>235(43.2)</td>
</tr>
<tr>
<td>High-risk</td>
<td>86</td>
<td>101</td>
<td>187(33.4)</td>
</tr>
<tr>
<td>Low-risk</td>
<td>38</td>
<td>85</td>
<td>123(22.0)</td>
</tr>
<tr>
<td>X</td>
<td>7(1.3)</td>
<td>3(5.2)</td>
<td>10(1.6)</td>
</tr>
<tr>
<td>High-risk</td>
<td>16</td>
<td>14</td>
<td>30(5.4)</td>
</tr>
<tr>
<td>Low-risk</td>
<td>6</td>
<td>4</td>
<td>10(1.8)</td>
</tr>
</tbody>
</table>

115
Figure 1. Age-specific prevalence of cervical human papillomavirus (HPV) DNA among 618 women. Mombasa, Kenya, 2003-2004.
Table 2. Detection of cervical HPV DNA according to sexual habits, reproductive characteristics and contraceptive use among 618 women. Mombasa, Kenya, 2003-2004.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>No of women</th>
<th>HPV DNA pos. (%)</th>
<th>OR(^1) 95% CI</th>
<th>OR(^2) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>145</td>
<td>59 (40.7)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>25-34</td>
<td>176</td>
<td>91 (51.7)</td>
<td>1.6</td>
<td>1.0-2.4</td>
</tr>
<tr>
<td>35-44</td>
<td>165</td>
<td>78 (47.3)</td>
<td>1.3</td>
<td>0.8-2.1</td>
</tr>
<tr>
<td>≥45</td>
<td>132</td>
<td>48 (36.4)</td>
<td>0.8</td>
<td>0.5-1.4</td>
</tr>
<tr>
<td>(\chi^2) for trend:</td>
<td></td>
<td></td>
<td>(p = 0.37)</td>
<td>(p = 0.16)</td>
</tr>
<tr>
<td>Age at sexual debut (yrs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-19</td>
<td>176</td>
<td>81 (46.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&lt;17</td>
<td>179</td>
<td>88 (49.2)</td>
<td>1.2</td>
<td>0.8-1.8</td>
</tr>
<tr>
<td>(\chi^2) for trend:</td>
<td></td>
<td></td>
<td>(p = 0.30)</td>
<td>(p = 0.24)</td>
</tr>
<tr>
<td>Lifetime number of sexual partners</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0-)1</td>
<td>311</td>
<td>132 (42.4)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>68 (50.4)</td>
<td>1.4</td>
<td>0.9-2.0</td>
</tr>
<tr>
<td>3-4</td>
<td>66</td>
<td>36 (54.6)</td>
<td>1.6</td>
<td>0.9-2.8</td>
</tr>
<tr>
<td>≥5</td>
<td>19</td>
<td>7 (36.8)</td>
<td>0.8</td>
<td>0.3-2.1</td>
</tr>
<tr>
<td>(\chi^2) for trend:</td>
<td></td>
<td></td>
<td>(p = 0.20)</td>
<td></td>
</tr>
<tr>
<td>Sexual partners in the last 12 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>104</td>
<td>52 (50.0)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>321</td>
<td>148 (46.1)</td>
<td>0.8</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td>≥2</td>
<td>24</td>
<td>13 (54.2)</td>
<td>1.1</td>
<td>0.4-2.6</td>
</tr>
<tr>
<td>(p = 0.55)</td>
<td>(p = 0.48)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Husband’s extramarital sexual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>relationships</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>138</td>
<td>53 (38.4)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Uncertain/don’t know</td>
<td>136</td>
<td>58 (42.7)</td>
<td>1.2</td>
<td>0.7-1.9</td>
</tr>
<tr>
<td>Yes</td>
<td>145</td>
<td>72 (49.7)</td>
<td>1.6</td>
<td>1.0-2.5</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age, as appropriate. \(^2\)Adjusted for age and lifetime number of sexual partners, as appropriate.
HPV = human papillomavirus; OR = odds ratio; CI = confidence interval.
3.3. HIV and cervical cancer in Kenya.

Gichangi P, De Vuyst H, Estambale B, Rogo K, Bwayo J, Temmerman M.

Article

HIV and cervical cancer in Kenya


*Department of Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya
bInternational Center for Reproductive Health, Ghent University, Ghent, Belgium
cDepartment of Medical Microbiology, University of Nairobi, Nairobi, Kenya
dNairobi Oncology Center, Nairobi, Kenya

Received 25 June 2001; received in revised form 19 September 2001; accepted 26 September 2001

Abstract

Objectives: To determine the effect of the HIV epidemic on invasive cervical cancer in Kenya. Methods: Of the 3902 women who were diagnosed with reproductive tract malignancies at Kenyatta National Hospital (KNH) from 1989 to 1998, 85% had invasive cervical cancer. Age at presentation and severity of cervical cancer were studied for a 9-year period when national HIV prevalence went from 5% to 5–10%, to 10–15%. Results: There was no significant change in either age at presentation or severity of cervical cancer. Of the 118 HIV (5%) women who were tested for HIV, 36 (31%) were seropositive. These women were 5 years younger at presentation than HIV-negative women. Conclusions: A two- to three-fold increase in HIV prevalence in Kenya did not seem to have a proportional effect on the incidence of cervical cancer. Yet, HIV-positive women who presented with cervical cancer were significantly younger than HIV-negative women. © 2002 International Federation of Gynecology and Obstetrics. All rights reserved.

Keywords: HIV; Cervical cancer; Kenya

1. Introduction

Cervical cancer is one of the AIDS-associated or AIDS-defining illnesses [1,2]. This is based on
HIV/AIDS include Kaposi sarcoma, non-Hodgkin's lymphoma (NHL), and childhood leiomyosarcoma [7–9].

Other studies have suggested a significant association between pre-malignant cervical lesions and HIV infection [10–12], and the association of HIV infection and cervical dysplasia has been confirmed [12]. Yet, the correlation between HIV infection and invasive cervical cancer remains inconclusive [3,6,13]. Reports on iatrogenically-induced immunosuppression to prevent rejection of transplanted organs clearly show an increased risk of cervical dysplasia and cervical cancer, however, suggesting a relationship between immunosuppression and cervical cancer [14,15].

If HIV infection increases both the likelihood of cervical cancer developing from dysplasia and the likelihood of a worsening of the stage of cervical cancer, a correlation could be expected between the rising HIV prevalence in Kenya and the incidence of cervical cancer.

This retrospective study was undertaken to examine the effects of increasing HIV prevalence in Kenya on patient age at presentation, and on the incidence and severity of invasive cervical cancer.

2.Materials and methods

Most patients treated for reproductive-tract cancers in the Radiotherapy Department of Kenyatta National Hospital (KNH), Nairobi, Kenya, are referred by the hospital's Department of Obstetrics and Gynecology. Others are referred from other hospitals. There are three cervical cancer treatment centers in Kenya: Kenyatta National Hospital (public) and Nairobi Hospital (private) in Nairobi, and Nyanza Provincial General Hospital (public) in the western part of the country.

A private hospital, Nairobi Hospital is not financially accessible to the majority of the Kenyan population. Nyanza Provincial General Hospital is not very operational because of a lack of qualified staff and adequate equipment. KNH therefore remains the national cervical cancer treatment center. It functions as a teaching as well as a referral hospital.

The number of patients admitted at Kenyatta National Hospital and the number of women with reproductive-tract cancer were obtained from the hospital's annual reports. All women diagnosed with reproductive-tract cancers in the departments of Obstetrics and Gynecology and Radiotherapy were included. Case records of patients with cervical cancer between 1989 and 1998 were retrieved from the Records Department at KNH.

At KNH, records not in use are usually destroyed after 10 years. All available records of patients with a diagnosis of reproductive-tract malignancies were assessed. Cases with a histologically-verified diagnosis of invasive cervical cancer were included. Cases with clinical diagnosis of invasive cancer but without histological results were reviewed by a gynecologist (P.G.) and were classified as either probable cases or unlikely cases of invasive cervical cancer. Probable cases of invasive cervical cancer were defined as cases with both a history suggestive of cervical cancer and a clinical examination highly suggestive of cervical cancer, but without histological confirmation. Unlikely cases of invasive cervical cancer were defined as cases with a history suggestive of invasive cervical cancer but an inconclusive clinical examination in the absence of histological results. Sociodemographic characteristics, number of pregnancies, history of Pap-smear testing, HIV serostatus, and International Federation of Gynecology and Obstetrics (FIGO) staging of cervical cancer — including histological subtypes and differentiations — were obtained from the case records of women with reproductive-tract malignancies.

2.1. Statistical methods

Data generated from the case records were coded and completed the questionnaire. They were then processed with statistical package SPSS, version 9.0 (SPSS Inc. Chicago, IL, USA). Analysis was stratified into three periods, 1989–90, 1991–94, and 1995–98, with respective HIV
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Cervix</td>
<td>401</td>
<td>84.7</td>
<td>398</td>
<td>85.5</td>
<td>378</td>
<td>92.8</td>
<td>210</td>
<td>86.4</td>
</tr>
<tr>
<td>Uterus</td>
<td>6</td>
<td>1.2</td>
<td>12</td>
<td>2.5</td>
<td>12</td>
<td>2.9</td>
<td>4</td>
<td>1.6</td>
</tr>
<tr>
<td>Ovary</td>
<td>54</td>
<td>11.4</td>
<td>38</td>
<td>8.1</td>
<td>24</td>
<td>9.8</td>
<td>26</td>
<td>7.1</td>
</tr>
<tr>
<td>Placenta</td>
<td>6</td>
<td>1.2</td>
<td>8</td>
<td>1.9</td>
<td>3</td>
<td>1.2</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Vagina/vulva(a)</td>
<td>12</td>
<td>2.5</td>
<td>11</td>
<td>2.3</td>
<td>9</td>
<td>2.2</td>
<td>2</td>
<td>0.8</td>
</tr>
<tr>
<td>Total new female cancers</td>
<td>473</td>
<td>465</td>
<td>472</td>
<td>243</td>
<td>368</td>
<td>459</td>
<td>321</td>
<td>419</td>
</tr>
<tr>
<td>New gynecology patients</td>
<td>1800</td>
<td>2218</td>
<td>1095</td>
<td>609</td>
<td>1082</td>
<td>1444</td>
<td>1443</td>
<td>1790</td>
</tr>
</tbody>
</table>

Data for 1990 and 1993 were incomplete. The table based on hospital reports.
\(a\)Coding for disease processes changed in 1997 from International Classification of Diseases 9 (ICD 9) to ICD 10.
prevalence of 5%, 5–10%, and 10–15% in women of reproductive age [16]. The number of cervical cancer cases, age at presentation, and the severity of cervical cancer were compared with HIV prevalence rates for women, using Yates corrected Chi-square test. Differences mean ages were tested using Student’s t-test.

3. Results

3.1. Magnitude of invasive cervical cancer

Of the 3902 women who were diagnosed with reproductive-tract malignancies at Kenyatta National Hospital (KNH) from 1989 to 1998, 3327 (85%) had invasive cervical cancer. Seventy six percent (2542/3327) of the case records reporting cervical cancer were retrieved for review, and 2382 (94%) of those were indicative of invasive cervical cancer. The other case records (26%) could not be found because of misfiling or loss.

As shown in Table 1, the number of cervical cancer cases and the number of new gynecology cases did not change significantly over an 8-year period (data for 1990 and 1993 were missing). Similarly, there was no significant change in proportion between cervical and other female reproductive-tract cancers between 1989 and 1998 (84% vs. 82%, \(P = 0.458\)).

3.2. Demographic characteristics of women

The mean age at diagnosis of cervical cancer was 47 ± 12 years, with approximately 30% of the women between 40 and 49 years. The mean age at presentation varied from 45 ± 13 years in 1989 to 47 ± 13 years in 1998 (\(P = 0.256\)). As shown in Table 2, the proportion of women with cervical cancer below 35 years did not change significantly over this period. Overall, women in Stage I to Stage IIa were 3 years younger than those in Stage IIb to Stage IV (45 ± 12 years vs. 48 ± 12 years, \(P < 0.001\)).

The 26% of women with secondary-school education or higher (51/195) were more likely to present with Stage I to Stage IIa than the 14% with lower education (240/1662, \(P < 0.001\)).

Among women with invasive cervical cancer, there was a significant decrease in the proportion of women with high parity (parity > 5), from 74% in 1989–90 to 68% in 1995–98, (\(P = 0.002\)) (Table 2). Nulliparity was rare in this group (2%). Most patients were ‘grandes multiparae’ (72%). In addition, high-parity women were significantly more likely to have Stage IIb–IV than women of lower parity.

Table 2

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV positive</td>
<td>6/23</td>
<td>12/34</td>
<td>18/61</td>
</tr>
<tr>
<td>Mean age ± S.D. (years)</td>
<td>47 ± 13</td>
<td>47 ± 12</td>
<td>47 ± 13</td>
</tr>
<tr>
<td>Age &lt; 35 years</td>
<td>21</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>331</td>
<td>697</td>
<td>671</td>
</tr>
<tr>
<td>Married</td>
<td>75.9</td>
<td>73.3</td>
<td>69.1</td>
</tr>
<tr>
<td>Single</td>
<td>4.3</td>
<td>5.6</td>
<td>8.7</td>
</tr>
<tr>
<td>Other</td>
<td>19.7</td>
<td>20.9</td>
<td>21.2</td>
</tr>
<tr>
<td>Previous Pap test</td>
<td>19/271</td>
<td>31/827</td>
<td>52/734</td>
</tr>
<tr>
<td>Parity</td>
<td>2.1</td>
<td>1.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Nullipara</td>
<td>9/437</td>
<td>14/921</td>
<td>18/960</td>
</tr>
<tr>
<td>Para 1–4</td>
<td>103/437</td>
<td>211/921</td>
<td>283/960</td>
</tr>
<tr>
<td>Para 5+</td>
<td>325/437</td>
<td>696/921</td>
<td>659/960</td>
</tr>
<tr>
<td>S.D., standard deviation.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3
Clinical FIGO staging of invasive cervical cancer over time

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>4% 9% 8% 6% 6% 8% 5% 6% 2% 4%</td>
<td>A 0 1 0 0 1 1 0 0 0 0</td>
<td>B 5 26 21 16 12 15 11 13 4 11</td>
<td>Stage II</td>
<td>45% 34% 34% 35% 39% 30% 33% 41% 28% 32%</td>
<td>A 11 23 19 23 16 11 20 32 15 19</td>
<td>B 47 85 68 64 77 53 57 61 46 75</td>
<td>Stage III</td>
<td>32% 42% 45% 44% 42% 42% 39% 39% 42% 36%</td>
<td>A 8 28 16 18 23 21 26 13 15 35</td>
</tr>
</tbody>
</table>

**Abbreviations:** CA CX, invasive cervical cancer; N, total case records reviewed.

Parity (86% vs. 82%, *P = 0.024*). Approximately 77% (1832/2382) of all case records showed Pap-smear history, and only 6% of them (102/1832) indicated that a Pap-smear had ever been done.

Of the 2382 patients with invasive cervical cancer, 118 (5%) had been tested for HIV, 36 of whom (31%) were HIV seropositive. The number of women tested for HIV did not differ between the periods 1989–90, 1991–94, and 1995–98 (Table 2). Women tested for HIV were significantly younger (42 ± 11 vs. 47 ± 12 years, *P < 0.001*). HIV-positive women were 5 years younger than HIV-negative women at the time of invasive cer-

### Table 4
Clinical and histological classification of invasive cervical cancer according to HIV seroprevalence rates in women

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>32</td>
<td>7.7%</td>
<td>6</td>
<td>7.3%</td>
<td>40</td>
<td>4.7%</td>
<td>0.004</td>
</tr>
<tr>
<td>Stage II</td>
<td>166</td>
<td>40.1%</td>
<td>331</td>
<td>37.1%</td>
<td>325</td>
<td>38.2%</td>
<td>0.091</td>
</tr>
<tr>
<td>Stage III</td>
<td>175</td>
<td>42.3%</td>
<td>414</td>
<td>46.4%</td>
<td>377</td>
<td>44.4%</td>
<td>0.032</td>
</tr>
<tr>
<td>Stage IV</td>
<td>40</td>
<td>9.6%</td>
<td>81</td>
<td>9.1%</td>
<td>107</td>
<td>12.6%</td>
<td>0.357</td>
</tr>
<tr>
<td>Stage I–Ha</td>
<td>66</td>
<td>15.9%</td>
<td>135</td>
<td>15.1%</td>
<td>126</td>
<td>14.8%</td>
<td>0.880</td>
</tr>
<tr>
<td>Stage IIb–IV</td>
<td>347</td>
<td>84.0%</td>
<td>757</td>
<td>84.8%</td>
<td>723</td>
<td>85.1%</td>
<td>0.519</td>
</tr>
<tr>
<td>Histological type</td>
<td>(N = 332)</td>
<td>(N = 721)</td>
<td>(N = 719)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell cancer</td>
<td>274</td>
<td>82.3%</td>
<td>619</td>
<td>85.8%</td>
<td>637</td>
<td>88.5%</td>
<td>0.026</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>17</td>
<td>5.1%</td>
<td>36</td>
<td>4.9%</td>
<td>41</td>
<td>5.7%</td>
<td>0.685</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>1</td>
<td>0.3%</td>
<td>4</td>
<td>0.5%</td>
<td>2</td>
<td>0.2%</td>
<td>--</td>
</tr>
<tr>
<td>Anaplastic</td>
<td>40</td>
<td>12.0%</td>
<td>62</td>
<td>8.5%</td>
<td>38</td>
<td>5.2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Differentiation of squamous cell carcinoma</td>
<td>(N = 159)</td>
<td>(N = 425)</td>
<td>(N = 435)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>18</td>
<td>11.3%</td>
<td>59</td>
<td>13.8%</td>
<td>63</td>
<td>14.4%</td>
<td>0.018</td>
</tr>
<tr>
<td>Moderate</td>
<td>49</td>
<td>30.8%</td>
<td>127</td>
<td>29.8%</td>
<td>133</td>
<td>30.5%</td>
<td>0.957</td>
</tr>
<tr>
<td>Poor</td>
<td>90</td>
<td>56.6%</td>
<td>239</td>
<td>56.2%</td>
<td>239</td>
<td>54.9%</td>
<td>0.154</td>
</tr>
</tbody>
</table>
3.3. Severity of invasive cervical cancer: clinical (FIGO) staging

Ninety percent of the cases of invasive cervical cancer were clinically staged, of which 6.4% were Stage I, 38.2% Stage II, 44.8% Stage III, and 10.6% Stage IV. Stage I accounted for less than 10% of the cases for any given year, with a range of 2–9% (Table 3). The proportion of women from Stage I to Stage IIa (13% in 1989 vs. 12% in 1998), or Stage IIb to Stage IV (87% in 1989 vs. 88% in 1998), did not change over time.

3.4. Cervical cancer histological subtypes

Of the 74% (1772/2382) histologically verified cases of invasive cervical cancer, squamous cell carcinoma was the most common histological subtype (1530/1772, 86%). Adenosquamous cancer was rare (7/1772, 0.4%), while adenocarcinoma represented 5% (95/1772) and anaplastic cervical cancers 8% (140/1772) of all invasive cervical cancers. The proportion of squamous cell carcinoma significantly increased from 83% in 1989–90 to 89% in 1995–98 ($P = 0.026$), with a concurrent decrease (from 12% to 5%, $P < 0.001$) in the proportion of cases reported as anaplastic (Table 4).

Histological differentiation was reported in 69% (1223/1772) of all histologically verified cases. Overall, approximately 48% of the histological subtypes were reported as poorly differentiated, 28% as moderately-well differentiated, 13% as well-differentiated and 11% as anaplastic. As shown in Table 4, cases of well-differentiated squamous cell carcinoma increased from 6% in 1989–90 to 15% in 1995–98 while moderately and poorly differentiated types did not differ over the same periods.

4. Discussion

Kenya is one of the sub-Saharan countries ravaged by the HIV/AIDS epidemic. By 1999, it was estimated that two million Kenyans were infected with the HIV virus [6]. In 1998, the prevalence rate of HIV infection among adults in Kenya was 15% [6]. Of the two million cases, one million were female (ratio of men/women in Kenya is 1:1).

Several cancers have been designated as AIDS-associated, with a significantly increased risk among HIV seropositive individuals [1,2,7,9]. Cervical cancer is gradually recognized as a sexually transmitted disease. The sexually transmitted etiological agent has been identified as the human papillomavirus HPV. Women with HIV infection are more likely to have a concurrent HPV infection [11,17]. There is firm evidence, however, that HIV is independently associated with an increased risk for cervical intra-epithelial neoplasms [10–12]. Some studies suggest that HIV infection is associated with the rapid progression of HPV-related cervical pre-malignant lesions to invasive cervical cancer or to advanced invasive cervical cancer [4,18].

In Kenya, the incidence of cervical cancer was estimated at 45/100,000 in the early 1980s. Over the next 10 years, the proportional incidence of invasive cervical cancer among new gynecology patients or among women with reproductive-tract cancer did not significantly change. HIV prevalence, however, increased two- to three-fold over the same period in women of reproductive age. These data should be interpreted with caution, as many confounders may have masked the relationship of cervical cancer and HIV. Some of the confounders could include different referral patterns and vertical intervention programs such as screening for cervical cancer. Yet, there is no evidence of change in the referral system for that period in Kenya, and no intervention program that could have influenced the number of reported or treated cases. Cost sharing (user fees), which was introduced in 1989, did not significantly decrease the number of patients’ visits at KNH.

In Uganda, Parkin et al. [9] reported a marked increase in the incidence of cervical cancer since 1960, with stabilization in 1990. In Harare,

Unlike cervical cancer, the incidence of Kaposi sarcoma seems to mirror the incidence/prevalence of HIV [7,9]. Kaposi sarcoma incidence significantly increased in HIV-infected individuals [20]. If HIV infection indeed shortens the latent period observed in the progression from premalignant cervical lesions to invasive disease, then women with HIV infection and invasive cervical cancer are expected to be younger than women with invasive cervical cancer only. Moreover, as HIV infection is much more common among young people, one could expect an earlier onset of the process of cervical cancer in this group. In Kenya, the peak age for HIV infection among women is 25–29 years. Our retrospective study shows that, over the 9 years considered, the mean age of women presenting with invasive cervical cancer remained stable at 47 years whereas the prevalence of HIV in women increased two- to three-fold. Similar observations have been reported in Zimbabwe [7] and Uganda [9] cancer-registry studies. The apparent absence of change in age of presentation could be due to several factors. One hypothesis is that HIV-infected women may die from HIV-related opportunistic infections before they develop invasive cervical cancer. The mean survival time for women with HIV infection in Kenya is 5 years [16].

Our study did show that among women tested for HIV infection, HIV-seropositive women with invasive cervical cancer were 5 years younger than HIV-negative women with invasive cervical cancer. The number of women tested for HIV in our study was low (5%), and they were significantly younger than those who were not tested, suggesting a selection bias in HIV testing. HIV testing is not a standard protocol at KNH. Because of the nature of our study design, it was not possible to know why tests were done. Our findings, however, are similar to those of Lomalisa et al. [22] who reported that HIV-seropositive women in South Africa presented with invasive cervical cancer 10 years earlier than HIV-negative women. Among the 5% women tested for HIV in our study, 31% were HIV positive. This is higher than the 2.9% reported by Rogo and Kavoo [23] in Kenya, the 7.5% reported by Lomalisa et al. [22], and the 12.6% reported by Sitas et al. in South Africa [6] among women with invasive cervical cancer.

The FIGO clinical staging and histological subtypes were used to assess the severity of cervical cancer. Our finding 6.4% of the women in Stage I is close to the 7% reported by Rogo et al. in 1990 using data from 1968 to 1979 from the same hospital [24]. The proportion of poorly-differentiated subtypes to well-differentiated cervical cancers did not significantly change over time. Our data show no change in the severity of cervical cancer over time at KNH, suggesting a lack of correlation between HIV prevalence in women and severity of cervical cancer. This observation is to be taken with caution as the HIV status of the women was unknown in most cases. Comparing time periods with different HIV infection prevalence, or with no HIV infection, may therefore be a very crude indicator.

The lack of correlation between HIV infection prevalence in women and mean age of presentation, proportional incidence of cervical cancer and severity of cervical cancer could be due to different reasons. HIV infection among women with cervical cancer may be an indicator of risky sexual behavior or shared risk factors. The fate of untreated HIV-related pre-malignant cervical lesions is unknown. One hypothesis is that HIV-infected women probably die before they develop invasive cervical cancer. To date, this hypothesis has not been proven in a thorough clinical study in Kenya. The lack of correlation is most likely due to prevalent opportunistic infections such as tuberculosis; a result of the unavailability of antiretroviral therapy, these infections may shorten the survival time of HIV-positive women. It is
also possible that the number of HIV-infected women with invasive cervical cancer may be too low to influence the mean age of presentation and the total number of cervical cancer cases.

In conclusion, despite a probable selection bias in HIV testing, our study suggests that HIV-seropositive women with cervical cancer present significantly younger than HIV-negative patients. This study did not show an increase in the proportion of invasive cervical cancer among the reproductive-tract cancers of women, nor did it show changes in mean age of presentation or severity of invasive cervical cancer over time. HIV prevalence has increased over time, however, doubling and tripling among women of reproductive age. Further studies are needed to determine the relationship of HIV infection and invasive cervical cancer.

Acknowledgements

The study was supported by the VLIR (Flemish Interuniversity Council), Belgium.

References


3.4. Impact of HIV infection on invasive cervical cancer in Kenyan women.


Impact of HIV infection on invasive cervical cancer in Kenyan women

Peter B. Gichangi, Job Bwayo, Benson Estambale, Hugo De Vuyst, S. Ojwang, Khamia Rogo, H. Abwao and Marleen Temmerman

Objectives: To determine the association between invasive cervical cancer (ICC) and HIV infection in Kenyan women.

Study design: Case-control, with ICC patients as cases, and women with uterine fibroids as controls.

Methods: Medical and socio-demographic data were collected from 367 ICC patients, and 226 women with fibroids. After informed consent, HIV testing was done.

Results: ICC patients were older than fibroid patients (48 versus 41 years; P<0.001), with an HIV seroprevalence of 15% and 12% respectively (P>0.05). However, cases younger than 35 years were 2.6-times more likely to be HIV positive than controls of similar age (35% versus 17%; odds ratio (OR), 2.6; P=0.043). ICC HIV-seropositive patients were, on average, 10 years younger than HIV-seronegative patients (40 versus 50 years; P<0.001). Eighty per cent of HIV-seropositive and 77% of HIV-seronegative ICC patients were in FIGO stage IIb or above. However, the odds of having poorly differentiated tumours was three times higher for HIV-seropositive than for HIV-seronegative ICC patients (77% versus 52%; OR, 3.1; P=0.038) after adjusting for histological cell type and clinical stage. Mean CD4 cell count was 833×10^6 cells/l in ICC and 1025×10^6 cells/l in fibroid patients (P=0.001).

Conclusion: Young women with ICC were more often HIV infected than women with fibroids of the same age groups. HIV infection was associated with poor histological differentiation of the tumours. These findings suggest an accelerated clinical progression of premalignant cervical lesions to ICC in HIV-infected women.

© 2003 Lippincott Williams & Wilkins

Keywords: invasive cervical cancer, HIV, seroprevalence, CD4, Kenya

Introduction

Cervical cancer is now recognized as a sexually transmitted disease because of the causal role of a sexually transmitted aetiologic agent, human papilloma virus (HPV), and the association of cervical cancer with sexual behaviour [1]. From 1973 to 1992, cervical cancer was the most common cause of sexually transmitted disease (STD)-related deaths among women in the USA with almost twice the number of deaths attributed to invasive cervical cancer (ICC) as to HIV infection [2]. Risk factors for cervical cancer include early coitarche, multiple sex partners, smoking, history of STD and immunosuppression [3]. Although the association of HIV infection and premalignant cervical lesions is now well established [4–9], data on the role of HIV in ICC is scanty [7,10] or
lacking [11–13]. The apparent association of HIV infection and ICC is of concern because in many areas with high incidence of cervical cancer, large numbers of women are also likely to be HIV infected, raising a theoretical possibility that HIV infection may increase the incidence of cervical cancer. Moreover, ICC and premalignant cervical lesions may increase transmission and acquisition of HIV infection. There are few studies from developing countries examining the relationship of HIV infection and ICC [14–17]. With limited resources for treatment of HIV infection, it is possible that HIV-infected women may die early before they develop ICC. However, if HIV infection causes rapid progression of premalignant cervical lesions [18–20], women with ICC may present earlier than usual [14,15] with possibly more advanced disease [10]. It is a common clinical impression that HIV-infected women with cervical cancer present at earlier ages [21,22]. In Kenya, HIV infection and ICC are both very prevalent. This study was undertaken to determine the association between ICC and HIV infection in Kenyan women.

Materials and methods

A prospective case–control design was used. Patients were recruited from radiotherapy unit, obstetrics and gynaecology wards of Kenyatta National Hospital (KNH) from January 2000 to March 2002. KNH is a national referral and teaching hospital for the University of Nairobi. Patients accessing the hospital are self-referral from Nairobi city, and referred patients from the whole of Kenya. KNH is the only public facility offering radiotherapy treatment in Kenya. Cases were cervical cancer patients seeking radiotherapy treatment at KNH while controls were consecutive women with a clinical diagnosis of uterine fibroids confirmed by ultrasound. Over 98% of all eligible patients were recruited. All enrolled participants received HIV pre-test and post-test counselling. A blood sample was obtained for HIV testing. HIV screening was done using the enzyme-linked immunosorbent assay (ELISA) (Biochem Immuno Systems Kit, Montreal, Quebec, Canada) and positive samples were confirmed using double ELISA (Biotech Ltd, Cambridge, Ireland). Blood was obtained for CD4 cell count using flow cytometry (FAC scan, Becton–Dickson) from 137 consecutive ICC and 81 fibroid patients. The number of patients tested for CD4 cell count was limited because of limited funding. Study staff administered a structured questionnaire. Federation International Gynaecology Obstetrics (FIGO) 1999 [23] clinical staging, histological subtypes and grading (scale of 1 to 3) were extracted from case records and histopathological reports. The pathologist and gynaecologists were not aware of the HIV serostatus of the patients.

Statistical analysis was carried out using SPSS version 10.0 (SPSS Inc. Chicago, Illinois, USA) statistical package. Fisher exact test and Yates corrected Chi-square testing was used to compare proportions. Differences between means were tested by t test. Odds ratio (OR) or adjusted OR (AOR) and their 95% confidence intervals (CI) were used to measure strength of associations. Multivariate logistic regression models included variables significant in univariate analysis or those that were thought to have biological influence on the dependent variable. Associations with two-sided P < 0.05 were considered statistically significant.

All study participants gave an informed consent. The KNH ethics and research committee and the University of Nairobi approved the study.

Results

Three hundred and sixty-seven ICC patients, and 226 women with uterine fibroids were recruited. The seven ICC and four fibroid patients who declined to be enrolled were not different in terms of age or clinical stage from those enrolled in the study. ICC patients were older than fibroid patients (48 ± 12 versus 41 ± 9 years; P < 0.001). Forty-six of the 364 (12.6%) ICC and 59 of 226 (26.1%) fibroid patients were < 35 years old. Overall HIV seroprevalence was 15% in cases and 12% in controls (P > 0.05). This crude comparison is probably misleading due to confounding by age. After controlling for age, marital status, lifetime sex partners and past STD, the odds of HIV infection was twice as large in cases than in controls (AOR 2.0; 95%CI, 1.1–3.5; P = 0.016).

Table 1 shows selected characteristics of cases (ICC patients) and controls (fibroid patients). Out of 358 ICC patients, 101 (28.2%) had no formal education as compared with 18 of 223 (8.1%) fibroids patients (OR, 4.5; P < 0.001). For both ICC and fibroid patients, the median number of pregnancies was 4.0 with mean of 5.6 ± 3.3 pregnancies. A Mann–Whitney test comparing parity of ICC and fibroid patients found P < 0.001. Mean parity was 6.4 ± 3.3 for ICC and 4.2 ± 2.9 for fibroid patients (t test, P < 0.001). Two hundred and fifty-seven of 367 (68.9%) ICC patients had five or more children as compared with 35% (80/226) of the fibroid patients (P < 0.001). Mean number of lifetime sex partners was 2.32 ± 1.64 for ICC and 2.59 ± 1.94 for fibroid patients, P = 0.078. The median number of lifetime sex partners for the study population was two. There was no significant difference in the proportion of women with more than two lifetime sex partners or those reporting previous history of STD between cases and controls.
Cervical cancer and HIV infection in Kenya Gichangi et al.

Table 1. Selected characteristics of cases (invasive cervical cancer patients, n = 367) and controls (fibroid patients, n = 226)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases [n (%)]</td>
<td>Controls [n (%)]</td>
</tr>
<tr>
<td>Age &lt; 35 years</td>
<td>46/364 (12.6)</td>
<td>59/226 (26.1)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>34/365 (9.3)</td>
<td>49/226 (21.7)</td>
</tr>
<tr>
<td>Low education</td>
<td>101/358 (28.2)</td>
<td>18/223 (8.1)</td>
</tr>
<tr>
<td>Parity &gt; 4</td>
<td>257/367 (68.9)</td>
<td>80/226 (35.4)</td>
</tr>
<tr>
<td>History of STD</td>
<td>44/359 (12.3)</td>
<td>35/223 (15.7)</td>
</tr>
<tr>
<td>Ever had a Papanicolaou smear</td>
<td>74/356 (20.8)</td>
<td>125/187 (66.8)</td>
</tr>
<tr>
<td>Lifetime sex partners (mean ± SD)</td>
<td>2.3 ± 1.6</td>
<td>2.6 ± 1.9</td>
</tr>
<tr>
<td>History of STD</td>
<td>37/316 (11.7)</td>
<td>16/166 (9.6)</td>
</tr>
<tr>
<td>Smokers</td>
<td>22/160 (6.3)</td>
<td>14/223 (6.3)</td>
</tr>
<tr>
<td>Condom use</td>
<td>52/356 (14.6)</td>
<td>31/226 (13.7)</td>
</tr>
</tbody>
</table>

Inclusion of parity and lifetime sex partners as continuous variable does not alter the conclusions from this table. STD, Sexually transmitted disease; OR, odds ratio; AOR, adjusted odds ratio; SD, standard deviation; CI, confidence interval.

Seventy-four of 356 (20.8%) ICC patients had had a Papanicolaou smear in their lifetime as compared to 125 of 187 (66.8%) fibroid patients (P < 0.001). On multivariate analysis controlling for age, i.e., < 35 years, marital status, lack of education, parity, Papanicolaou smear testing, lifetime sex partners, history of STD and HIV, the odds of being HIV infected was three times as large for ICC patients 34 years or younger as compared with fibroid patients of similar age (AOR, 3.3). Controls were more likely to have had a Papanicolaou smear test in the past than cases (AOR, 3.8).

Among the patients with fibroids, HIV-seronegative and -seropositive women were similar in age, marital status, level of education, previous history of STD and parity (% 4 or > 4) (data not shown). HIV-seropositive women with ICC were, on average, about 10 years younger as compared with fibroid patients of similar age (< 35 years, AOR, 0.4). There was no difference in marital status, level of education, history of STD and proportion of patients with more than two lifetime sex partners or mean number of sex partners between HIV-seropositive and -seronegative ICC patients. On multivariate analysis, including age (< 35 years), marital status, lack of education, parity, Papanicolaou smear testing, lifetime sex partners, history of STD, HIV seropositivity among ICC patients was significantly and independently associated with young age, < 35 years (AOR, 4.0) and lower parity, (four or fewer pregnancies) (AOR, 0.4).

Table 2. Correlates of HIV infection in invasive cervical cancer (ICC) patients (n = 365)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV positive [n (%)]</td>
<td>HIV negative [n (%)]</td>
</tr>
<tr>
<td>Age &lt; 35 years</td>
<td>16/53 (30.2)</td>
<td>30/309 (9.7)</td>
</tr>
<tr>
<td>Unmarried</td>
<td>8/53 (15.1)</td>
<td>26/303 (8.4)</td>
</tr>
<tr>
<td>No education</td>
<td>9/51 (17.6)</td>
<td>91/305 (29.8)</td>
</tr>
<tr>
<td>Parity (mean ± SD)</td>
<td>4.6 ± 2.6</td>
<td>6.7 ± 3.2</td>
</tr>
<tr>
<td>Ever had a Papanicolaou smear</td>
<td>16/51 (31.4)</td>
<td>58/303 (19.1)</td>
</tr>
<tr>
<td>More than two lifetime sex partners</td>
<td>21/46 (45.7)</td>
<td>104/391 (35.7)</td>
</tr>
<tr>
<td>Lifetime sex partners (mean ± SD)</td>
<td>2.5 ± 1.5</td>
<td>2.3 ± 1.7</td>
</tr>
<tr>
<td>History of STD</td>
<td>10/51 (19.6)</td>
<td>34/306 (11.1)</td>
</tr>
</tbody>
</table>

Inclusion of parity and lifetime sex partners as continuous variable does not alter the conclusions from this table. STD, Sexually transmitted disease; OR, odds ratio; AOR, adjusted odds ratio; SD, standard deviation; CI, confidence interval.

135
Three hundred and forty-one out of 365 (93.4%) of the ICC patients were clinically staged. Overall, 10% were in FIGO clinical stage I, 43% stage II, 42% stage III and 6% stage IV. Twenty-six of 50 (52%) HIV-seropositive women, were in stage II, 42% in stage III, while for HIV-seronegative women, about equal proportions, [41.2% (120/291) and 46.6% (121/291)] were in stage II and III respectively. Twenty per cent (10/50) of the HIV-seropositive patients were in stage I–IIa as compared to 23% (67/283) of the HIV-seronegative patients (P > 0.05). FIGO clinical stage of ICC was not influenced by educational status or age of the patient (<35 years) (Table 3). Three of 9% (3.1%) patients in stage III and above had CD4 cell count < 200 × 10^6 cells/l as compared to 6% (2/32) of the patients in stage I–IIa, (OR, 2.1; P = 0.429).

Histological cell typing reports were available for 79.4% (290/365) of the ICC patients. Squamous cell histological subtype was the most common accounting for over 79% (230/290) of the patients with known histology. The degree of differentiation was indicated in 53.8% (156/290) histological biopsy reports. Twenty of 26 (76.9%) HIV-seropositive as compared with 67 of 130 (51.5%) HIV-seronegative ICC patients had poorly differentiated tumours even after controlling for histological cell type and clinical stage (AOR, 2.9; P = 0.038). Only 3.4% (2/58) of the poorly differentiated histological subtypes had CD4 cell count < 200 × 10^6 cells/l as compared to well differentiated tumours, (OR, 0.5; 95% CI, 0.1–3.7; P = 0.492).

Table 3. HIV infection and invasive cervical cancer (ICC) tumour characteristics (n = 365)

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV positive [n (%)]</th>
<th>HIV negative [n (%)]</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical (FIGO 1995) staging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3 (6.0)</td>
<td>31 (10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>26 (52.0)</td>
<td>120 (41.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>21 (42.0)</td>
<td>121 (41.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>19 (6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50 (100)</td>
<td>291 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I–IIa of ICC</td>
<td>10/50 (20.0)</td>
<td>67/283 (23.0)</td>
<td>1.2 (0.6–2.5)</td>
<td>0.637 1.4 (0.6–3.4) 0.408</td>
</tr>
<tr>
<td>Stage I–IIa of ICC among patients with no education</td>
<td>2/9 (22.2)</td>
<td>14/88 (15.9)</td>
<td>1.5 (0.3–8.1)</td>
<td>0.627</td>
</tr>
<tr>
<td>Stage I–IIa of ICC among patients &lt; 35 years old</td>
<td>5/16 (31.3)</td>
<td>6/27 (22.2)</td>
<td>1.6 (0.4–6.4)</td>
<td>0.512</td>
</tr>
<tr>
<td>Stage I–IIa of ICC among patients &gt; 35 years old</td>
<td>5/34 (14.7)</td>
<td>61/262 (23.3)</td>
<td>1.7 (0.7–4.7)</td>
<td>0.258</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>33 (78.7)</td>
<td>197 (79.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1 (2.3)</td>
<td>21 (8.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>0</td>
<td>1 (0.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaplastic</td>
<td>5 (11.6)</td>
<td>12 (4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>4 (9.4)</td>
<td>16 (6.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>43 (100)</td>
<td>247 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated tumours</td>
<td>20/26 (76.9)</td>
<td>67/130 (51.5)</td>
<td>3.1 (1.2–8.3)</td>
<td>0.017 2.9 (1.1–8.0) 0.038</td>
</tr>
</tbody>
</table>

OR, Odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

ICC patients had significantly lower mean CD4 cell count than fibroid patients (883 ± 354 versus 1006 ± 454 × 10^6 cells/l). Even after controlling for age, ICC patients had significantly lower CD4 cell count than fibroid patients. Overall, 3.6% (5/137) of the ICC patients had CD4 cell count < 200 × 10^6 cells/l, 4.4% (6/137) 200–350 × 10^6 cells/l, and 92% (126/137) > 350 × 10^6 cells/l. Seventeen per cent (4/23) HIV-seropositive ICC patients had CD4 cell count < 200 × 10^6 cells/l as compared with 0.9% (1/114) ICC HIV-seronegative patients. The number of HIV-seropositive cases was too small for further detailed stratified analysis. Five of the 81 (6%) fibroid patients had CD4 cell count < 200 × 10^6 cells/l, 2.5% (2/81) 200–350 × 10^6 cells/l, and 91.4% (74/81) > 350 × 10^6 cells/l (data not shown). Among HIV-seropositive patients, ICC and fibroid patients had similar CD4 cell counts (532 ± 320 × 10^6 cells/l versus 588 ± 463 × 10^6 cells/l; P = 0.673) while for HIV-seronegative patients, ICC patients had significantly lower CD4 cell count than fibroid patients (894 ± 331 × 10^6 cells/l versus 1079 ± 415 × 10^6 cells/l; P = 0.001).

Discussion

Our data show that ICC HIV-seropositive patients are 10 years younger at presentation than ICC HIV-seronegative patients, even after controlling for education, number of sex partners and previous history of STD. This could suggest that HIV infection may

AIDS 2003, Vol 17 No X
similar observations were reported from South Africa, Lomalisa et al. [15] found HIV prevalence of 7.6% in cervical patients. These observed differences among various studies could be accounted for by differences in HIV seroprevalence in the general population, as shown by the two South African studies from sites with different background HIV seroprevalence, 32.5% in KwaZulu Natal [24] and 23.9% in Gauteng province [29], or by the degree of immunosuppression. Our finding of mean CD4 cell count of 588 × 10^6 cells/l among HIV-infected women with ICC suggests that Kenyan cervical cancer patients are not as severely immunocompromised as South African women (mean CD4 cell count 316 × 10^6 cells/l) [15] or cervical cancer patients in the USA (mean CD4 cell count 362 × 10^6 cells/l) [19].

Our data does suggest a more rapid increase in HIV seroprevalence in ICC patients, than in the general population. The HIV seroprevalence in ICC patients has increased 10-fold from 1.5% in 1990 [30] to 15% in this study, as compared to a threefold increase in HIV seroprevalence from 5% in 1990 to current level of 14–15% in 2002 among Kenyan women of reproductive age [31]. Similar observations were reported from South African cervical cancer patients. Lomalisa et al. [15] observed doubling of HIV prevalence from 3.9% in 1995 to 7.2% in 2000, and Moodley et al. [24] reported quadrupling from 5 to 21%. ICC being an ulcerative condition may facilitate transmission and acquisition of HIV infection, which could account for the rapid increase in HIV prevalence in this population. Indeed, the odds of being HIV infected among ICC patients less than 35 years old was three times as large as that for women with uterine fibroids despite similar other risk factors for HIV transmission/acquisition in this study.

Outcome of ICC is dependent on FIGO clinical stage, age of the patient, tumour characteristics and treatment given. Five-year survival for stage I is 77–84%, stage II 54–67%, stage III 13–40% while for stage IV it is 5–13% [32]. HIV infection was associated with moderate to poorly differentiated histological subtypes, which is a poor prognostic factor [33]. Moodley et al. [24] reported that 37% of the HIV-seropositive patients had poorly differentiated tumours. HIV infection was not associated with advanced clinical stage at presentation in our study. Unlike in other studies [15, 19, 20], the lack of association of HIV infection and advanced stage of cervical cancer could be related to the relatively good immunostatus in our patients (mean CD4 cell count 532 × 10^6 cells/l) or to lack of power since only about 37% (137/367) had CD4 cell count determination due to cost constraints. Advanced stages of HIV disease have been associated with severe immunosuppression [15, 19, 20].

Major limitations of the study include the was use of recall data, such as lifetime sex partners and past STD, which could not be validated, and the inclusion of hospital patients only. ICC patients accessing hospital facilities may be a biased population. The majority of ICC patients were from the neighbourhood of the hospital suggesting limited access for patients far from the hospital. Early death of HIV-infected women before they develop ICC, or before they come to hospital due to other HIV related opportunistic infections could have resulted in underestimating the HIV seroprevalence among ICC patients in our study. Power of the study to determine the association of immunosuppression with stage of cervical cancer was low due to financial limitations of CD4 cell count determination. Another limitation of the study was the use of fibroid patients as a control group. Indeed, fibroid patients were different from ICC in terms of younger age, less likely to be married, both risk factors for HIV infection, more educated and to have had a Papanicolaou smear in the past. Despite these differences, ICC and fibroid patients were similar in mean number of lifetime sex partners and previous history of STD. The foregoing suggests that fibroid patients may not be comparable to ICC patients, thus interpretation of these results has to be done with those differences in consideration.

In conclusion, our data provide evidence for a higher HIV prevalence in women with ICC as compared to women of the same age group with uterine fibroids. Although HIV infection per se did not confer an
adverse effect on severity of ICC as assessed by FIGO clinical staging, it was associated with poor histological differentiation of the tumour, which is a poor prognostic factor for tumour spread and outcome of treatment and younger age at presentation. Further research is needed to examine the impact of the younger age at presentation of ICC HIV-seropositive patients and the poor histological differentiation as regards outcome of management of ICC.

Acknowledgements

The authors thank the director of Kenyatta National Hospital for allowing access to patients, and the staff in radiotherapy and obstetrics and gynaecology wards. We acknowledge S. Vansteelandt, Department of Applied Mathematics and Informatics, Ghent University, Belgium for assistance in data analysis. Special thanks go R. Kilonzo, J. Mbithi, the study staff, and laboratory technicians in Department of Medical Microbiology, University of Nairobi.

Sponsorship: Supported by the VLIR (Flemish Interuniversity Council), Belgium.

References

3.5. Human papillomavirus types in women with invasive cervical carcinoma by HIV status in Kenya.


Hugo De Vuyst¹, Peter Gichangi², Benson Estambale³, Eliud Njuguna⁴, Silvia Franceschi¹, Marleen Temmerman⁵

¹International Agency for Research on Cancer, Lyon, France.
²Department of Human Anatomy and Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya
³Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, Kenya
⁴Radiotherapy Unit, Kenyatta National Hospital, Nairobi, Kenya
⁵Department of Obstetrics and Gynecology, Ghent University, Ghent, Belgium

Running head: HPV type in invasive cervical cancer by HIV status

The references of this paper are listed at the end of this section.
ABSTRACT

In order to evaluate the fraction of invasive cervical carcinoma (ICC) that could be prevented in HIV-infected women by vaccines currently available against human papillomavirus (HPV)16 and 18, we conducted a cross-sectional study in women with ICC in Nairobi, Kenya. Fifty-one HIV-positive women were frequency-matched by age to 153 HIV-negative women. Cervical cells were tested for HPV-DNA using PCR-based assays (SPF10-INNO-LiPA). Comparisons were adjusted for multiplicity of HPV types. As expected, multiple-type infections were much more frequent in HIV-positive women (37.2%) than HIV-negative (13.7%) women, but the distribution of HPV types was similar. HPV-16 was detected in 41.2% vs. 43.8% and HPV 16 and/or 18 in 64.7% vs. 60.1% of HIV-positive vs. HIV-negative women, respectively. The only differences of borderline statistical significance were an excess of HPV-52 (19.6% vs. 5.2%) and a lack of HPV-45 (7.8% vs. 17.0%) in HIV-positive women compared to HIV-negative women, respectively. We have been able to assess an unprecedented number of ICCs in HIV positive women, but as we did not know the age of HIV acquisition, we cannot exclude that it had occurred too late in life to affect the type of HPV involved in cervical carcinogenesis. However, if our findings were confirmed, they would suggest that the efficacy of current vaccines against HPV16 and 18 to prevent ICC is similar in WHIV and in HIV-negative women, provided vaccination is given pre-puberty age, before sexual debut, as recommended.

Keywords: HIV, cervical cancer, human papillomavirus, polymerase chain reaction, Africa
INTRODUCTION

The main cause of invasive cervical carcinoma (ICC) is infection with high-risk human papillomavirus (HPV), most notably HPV16 and 18, which have been detected in approximately 70% of ICC in all world regions. Newly developed vaccines have been shown to be highly efficacious in preventing infection with these HPV types and routine vaccination of adolescent females is now being recommended. Women infected with HIV are at increased risk of HPV infection and development of precancerous and cancerous lesions of the cervix. Furthermore, a large systematic review of HIV-positive women with normal cytology, low-grade and high-grade intraepithelial lesions, has shown that HPV16 is relatively under-represented compared to HIV-negative women. This difference has been attributed to the fact that clearance of HPV16 is less dependent on an individual’s immune status than clearance of other high-risk types, as shown in HIV-positive women. Information on HPV types in ICC has been reported, however, in only 14 HIV-positive women. We have therefore carried out a comparison of the distribution of HPV types in ICC in women with and without HIV in Kenya, a country hit hard by the HIV epidemic (www.unaids.org/en/Regions_Countries/Countries/kenya.asp).

METHODS

Between January 2000 and March 2002, we identified and obtained informed consent from 367 women with ICC (i.e., 96% of those eligible) who presented themselves for treatment at the Kenyatta National Hospital, Nairobi, Kenya. HIV testing was performed using enzyme-linked immunosorbent assay (ELISA, Biochem Immuno Systems Kit, Montreal, Canada) and double ELISA (Biotech Ltd., Cambridge, Ireland) for confirmation. Exfoliated tumour cells were collected using a cervex brush (Rovers Medical Devices, Oss, the Netherlands) and stored at -20°C in phosphate buffered saline. Fifty-three women (14.4%) were HIV-positive, of whom 51 (mean age: 40.1; SD 11.0) had an adequate cell sample and were included in the present analysis. CD4 count was available for 41 women, of whom 20 had a CD4 count <500/µL, and seven a CD4 count <200/µL. HIV-positive women were frequency-matched by age to 153 HIV-negative women (mean age 45.0; SD 12.4). Medical records and histological diagnosis were
revised by one of the authors (Peter Gichangi). The vast majority of ICC was squamous cell carcinoma (95.1% and 92.6%, respectively, in women with and without HIV).

Samples were shipped to the Laboratory of Virology, Ghent University Hospital, Belgium, where HPV DNA was isolated by incubating the samples with proteinase K. Broad-spectrum HPV DNA amplification was performed with a short polymerase chain reaction (PCR) fragment (SPF10) primer set. The PCR product was analysed and genotyped directly on a HPV genotype Line Probe Assay (INNO-LiPA Version 2, Innogenetics, Ghent, Belgium), that detects 24 HPV types (6, 11, 16, 18, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 70, 74). A double-control line (generic probe) was present on the LiPA strip for confirmation of any mucosal HPV type. HPV amplimers which did not hybridise to any type-specific probe were assigned HPV genotype X (uncharacterised type). Each experiment was also controlled with separate positive and negative PCR controls.

The ethics and research committee of the Kenyatta National Hospital, the University of Nairobi, and the International Agency for Research on Cancer approved the study.

Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) of being positive for specific HPV type(s) by HIV status were computed separately among women positive for single-type and multiple-type infections, and overall, after adjustment for multiplicity of types, using the Mantel-Haenzel test.

RESULTS

Figure 1 shows the distribution of HPV types in single- and multiple-type infections found among HIV-positive and HIV-negative women. As expected, multiple-type infections were much more frequently detected in HIV-positive (37.2%) than HIV-negative women (13.7%). More than two HPV types were detected in seven HIV-positive and five HIV-negative women (data not shown). HPV16 was the type detected most frequently in both groups (41.2% of HIV-positive and 43.8% of HIV-negative women). HPV16 and/or 18 was found in 64.7% vs. 60.1%, respectively. The next most frequent types were HPV52 (19.6%), 35, 45 and 56 (7.8% each) among HIV-positive women, and HPV45 (17.0%), 35 and 52 (5.2% each) among HIV-negative women. Type X was detected in one HIV-positive and five HIV-negative women. Five women were HPV-negative, all of whom were also HIV-negative.
Table 1 shows the OR for the detection of the most common HPV types among HIV-positive compared to HIV-negative women. No significant differences in HPV type distribution by HIV status emerged in either single- or multiple-type infections or overall. Differences of borderline statistical significance emerged for HPV52 (excess) and HPV45 (lack) among HIV-positive women. For HPV52 but not for HPV45, the difference clearly emerged between HIV-positive and HIV-negative women with single-type infections.

DISCUSSION

Our present study, by far the largest comparison of HPV types in ICCs in women with and without HIV carried out to date, suggests that there is no substantial difference in the relative importance of HPV16 and 18 by HIV status. The slight excess of HPV52 among HIV-positive women with ICC is of some interest as it is clearly present in single-type infections and had already emerged in HIV-positive women with high-grade squamous intraepithelial lesions.

The comparison of HPV type distribution by HIV status is substantially complicated by the greater frequency of multiple-type infections in HIV-positive women. We have, however, carried out our HPV testing using a validated assay that has been shown to be very sensitive in detecting multiple-type infections, and we have confirmed our findings after stratification and adjustment for multiple infection. Nonetheless, half of the occurrences of HPV types 16 and/or 18 happen in multiple type infections among WHIV (Figure 1), and it is not known to date whether the remaining HR HPV types would have caused invasive disease in the absence of HPV 16 or 18.

A major weakness of our present study is the lack of information on year of seroconversion among HIV-positive women. CD4 count, although not always available, suggested that half had a CD4 count of ≥500/µL, which would suggest a relatively recent HIV infection. As pathogenesis of persistent HPV into ICC takes at least a decade, we should ideally focus on women who have acquired the cancer causing HPV type after HIV infection and have survived long enough to develop invasive cervical cancer. It is thus possible that HIV infection had occurred in our study women too late in life to affect the HPV type involved in cervical carcinogenesis, and that, as a result, our present findings are biased towards a lack of difference.
Therefore confirmation on larger series of ICC among women who are known, or likely to have been infected with HIV at an early age is needed.

ACKNOWLEDGEMENTS

We thank Dr Salvatore Vaccarella for statistical analysis, Ms Jayne Mbithi for performing the HPV testing and Ms Regina Kilonso for HIV counselling. Funding was provided by the Flemish Interuniversity Council (VLIR) and the Bill & Melinda Gates Foundation (grant number 35537).
REFERENCES


FIGURE LEGENDS

Figure 1. Prevalence of human papillomavirus (HPV) types in single- and multiple-type infections. Nairobi, Kenya, 2000-2002
Table 1: Prevalence of type-specific HPV infection among cervical carcinoma cases by HIV serostatus and single or multiple type infection. Nairobi, Kenia.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Single type infection</th>
<th></th>
<th>Multiple type infections</th>
<th></th>
<th></th>
<th>OR(95% CI)1</th>
<th>OR(95% CI)1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% on HIV+ Women (N=31)</td>
<td>% on HIV- Women (N=123)</td>
<td>OR(95% CI)1</td>
<td>% on HIV+ Women (N=19)</td>
<td>% on HIV- Women (N=20)</td>
<td>OR(95% CI)1</td>
<td>OR(95% CI)1,2</td>
</tr>
<tr>
<td>16</td>
<td>45.2</td>
<td>46.3</td>
<td>1.0(0.4-2.1)</td>
<td>36.8</td>
<td>50.0</td>
<td>0.6(0.2-2.1)</td>
<td>0.8(0.4-1.6)</td>
</tr>
<tr>
<td>18</td>
<td>12.9</td>
<td>17.9</td>
<td>0.7(0.2-2.1)</td>
<td>52.6</td>
<td>25.0</td>
<td>3.3(0.9-12.9)</td>
<td>1.3(0.6-2.9)</td>
</tr>
<tr>
<td>16 and/or 18</td>
<td>58.1</td>
<td>64.2</td>
<td>0.8(0.3-1.7)</td>
<td>79.0</td>
<td>65.0</td>
<td>2.0(0.5-8.5)</td>
<td>1.0(0.5-2.0)</td>
</tr>
<tr>
<td>31</td>
<td>3.2</td>
<td>0.8</td>
<td>4.1(0.2-66.9)</td>
<td>10.5</td>
<td>20.0</td>
<td>0.5(0.1-2.9)</td>
<td>0.8(0.2-3.7)</td>
</tr>
<tr>
<td>39</td>
<td>0.0</td>
<td>1.6</td>
<td>0</td>
<td>0.0</td>
<td>15.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>6.5</td>
<td>4.9</td>
<td>1.3(0.3-7.0)</td>
<td>10.5</td>
<td>10.0</td>
<td>1.1(0.1-8.4)</td>
<td>1.2(0.3-4.5)</td>
</tr>
<tr>
<td>45</td>
<td>12.9</td>
<td>13.8</td>
<td>0.9(0.3-3.0)</td>
<td>0.0</td>
<td>45.0</td>
<td>0</td>
<td>0.4(0.1-1.0)</td>
</tr>
<tr>
<td>52</td>
<td>9.7</td>
<td>1.6</td>
<td>6.5(1.0-40.6)</td>
<td>36.8</td>
<td>30.0</td>
<td>1.4(0.4-5.2)</td>
<td>2.8(0.9-8.6)</td>
</tr>
<tr>
<td>Any other than 16/18</td>
<td>41.9</td>
<td>35.8</td>
<td>1.3(0.6-2.9)</td>
<td>100.0</td>
<td>90.0</td>
<td>0</td>
<td>1.5(0.7-3.2)</td>
</tr>
</tbody>
</table>

1ORs are computed with the Mantel-Haenszel method. 2ORs are stratified by multiplicity of infection.

5 women among HIV- and 1 among HIV+ do not have the information on multiplicity (type X infection).
3.6. Comparison of pap smear, visual inspection with acetic acid, human papillomavirus DNA-PCR testing and cervicography.


Comparison of pap smear, visual inspection with acetic acid, human papillomavirus DNA-PCR testing and cervicography

H. De Vuysta, P. Claeysa, S. Njirub, L. Muchiric, S. Steyaertd, P. De Suttere, E. Van Marckf, J. Bwayob, M. Temmermanaf,

aInternational Centre for Reproductive Health, Ghent University, De Pintelaan, 185, B-9000 Ghent, Belgium
bDepartment of Medical Microbiology, University of Nairobi, Nairobi, Kenya
cDepartment of Human Pathology, University of Nairobi, Nairobi, Kenya
dDepartment of Clinical Biology, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium
eDepartment of Gynecology and Obstetrics, Gynecological Oncology Unit and Colposcopy Clinic, Free University of Brussels, Brussels, Belgium
fDepartment of Pathology, Antwerp University, Antwerp, Belgium

Received 3 August 2004; received in revised form 12 January 2005; accepted 12 January 2005

Abstract

Objective: To assess the test qualities of four screening methods to detect cervical intra-epithelial neoplasia in an urban African setting. Method: Six hundred fifty-three women, attending a family planning clinic in Nairobi (Kenya), underwent four concurrent screening methods: pap smear, visual inspection with acetic acid (VIA), PCR for high risk human papillomavirus (HR HPV) and cervicography. The presence of cervical intra-epithelial neoplasia (CIN) was verified by colposcopy or biopsy. Result: Sensitivity (for CIN2 or higher) and specificity (to exclude any CIN or cancer) were 83.3% (95% CI [73.6, 93.0]) and 94.6% (95% CI [92.6, 96.5]), respectively, for pap smear; 73.3% (95% CI [61.8, 84.9]) and 80.0% (95% CI [76.6, 83.4]) for VIA; 94.4% (95% CI [84.6, 98.8]) and 73.9% (95% CI [69.7, 78.2]) for HR HPV; and 72.3% (95% CI [59.1, 85.6]) and 93.2% (95% CI [90.8, 95.7]) for cervicography. Conclusion: The pap smear had the highest specificity (94.6%) and HPV testing...
1. Introduction

Screening for cervical lesions has proven successful in the industrialized world, with incidences of cervical cancer reduced by 80% in countries with organized screening and treatment programs [1]. The success of these programs can largely be attributed to the use of the Papanicolaou (pap) smear [2]. In the developing world, however, the incidence of cervical cancer remains high, particularly in sub-Saharan Africa, where cervical cancer is the most common cancer in women [3]. The lack of organized cervical cancer control programs is the main reason for this. Effective cytological screening programs are difficult to implement in low resource settings because of high cost, training requirements for laboratory technicians, and competing health priorities. Alternative screening methods are therefore being assessed. This paper reports on the test qualities of pap smear, visual inspection with acetic acid (VIA), cervicography, and DNA-PCR testing for human papillomavirus (HPV).

2. Materials and methods

Between January 1998 and July 2000, a cross-sectional study was carried out in non-pregnant women aged 25—55 attending a family planning clinic in Nairobi, Kenya. The study was approved by the National Ethical Review Committee of Kenyatta National Hospital, Nairobi. The staff consisted of a study nurse, who received a 4-day training in the technique of VIA with a pictorial atlas for visual inspection of the cervix [4] and projected images of cervices, followed by hands-on training, and two medical doctors trained in colposcopy.

Each working day, the first eight consecutive clients presenting at the family planning clinic were invited to participate in the study. A signed informed consent was obtained from all participants. The study nurse was blinded to the clinical background (referred patients or women attending for family planning). At the first visit (V1), exfoliative cervical cells were obtained using a Cervex Brush (Rovers Medical Devices, Oss, The Netherlands). One smear was made on a glass slide for staining according to the Papanicoloau method. The brush was then submerged and stirred in 10 ml phosphate buffered saline (PBS) and frozen at −20 °C. A VIA (VIA1) examination was then performed (inspecting the transformation zone) after application of commercially available vinegar (acetic acid 3—5%) for 2 min and illumination with a halogen torch. VIA findings were recorded by the study nurse in a clinical records form (CRF). Two photographic slides were taken with a specially designed 35 mm reflex camera (cervicography) (National Testing Laboratories, Fenton, USA). Serum samples were obtained for HIV testing after pretest counseling. Blood samples were tested for HIV-1 and HIV-2 using ELISA Detect HIV1/2 (Immunosystems, Montreal, Quebec) and Recombigen HIV1/2 (Trinity Biotech, Galway, Ireland). All the women were invited to come for a follow-up visit after 3 weeks (V2). At this visit, VIA (VIA2) was performed on all women by the study nurse and colposcopy by the doctor. After these examinations, the findings were registered in the CRF and the result of the pap smear disclosed to the examinators and communicated to the woman. If the pap smear, VIA2, or colposcopy was positive, a biopsy and/or endocervical curettage was obtained.

Pap smears and cervical biopsies were processed and analyzed at the Department of Human Pathology, University of Nairobi, Kenya. Cytology was reported according to the Bethesda 1991 classification. All positive smears and 60% of the negatives, randomly chosen, were sent to the Department of Pathology, Antwerp University, Belgium for quality review. A Cohen’s Kappa for agreement of 0.623 was found between the two institutions. The original Nairobi results before quality review were used in this analysis. All histology specimens were also reviewed and the Antwerp results were used for final data analysis. HPV samples were shipped to the Laboratory of Virology, Ghent University, Belgium for HPV DNA extraction, detection and genotyping, as described earlier [5]. Cervicography slides were assessed at the department of gynecology, Free University of Brussels, Belgium, by a senior staff colposcopist and accredited cervicography evaluator (PDS).

All examiners interpreting pap smears, HPV DNA PCR, or cervicographies were blinded to the clinical background and to other screening test results.
A pap smear was considered positive in case of LSIL or more severe lesions. VIA was considered positive in case of well defined, distinct acetowhite lesions on the cervix, close to the os. Colposcopy was considered positive for a colposcopic impression of LSIL or more. Any abnormal result on biopsy was taken as the final positive diagnosis, hereafter called the “reference test”. If biopsy was negative, or in case there was no indication for biopsy (all screening tests and colposcopy were negative), the reference test was considered negative. Lesions of CIN2 or worse on the reference test are considered diseased and reported as cases. The presence of any HPV DNA resulted in a positive HPV test, but we also assessed the presence of DNA from high-risk HPV types (HPV HR).

Cervicographies were reported using the classification criteria proposed by Schneider et al. [6]. All grades equal to or higher than a low grade lesion were considered as a positive test result.

Data were entered in Epi-info (CDC, USA; WHO, Geneva) and analyzed in SPSS 10.0.5 for windows (SPSS, Chicago, IL, USA). Comparisons of categorical variables were made using Pearson’s X2 and Fisher’s exact tests. Means of continuous variables were compared using the Independent Samples t-test. Exact 95% confidence intervals for diagnostic sensitivity (positive test results compared to the reference test) and specificity (negative test results compared to the reference test) were reported where appropriate.

3. Results

3.1. Characteristics of the study population

Of the 816 women presenting at Visit 1, 653 attended the clinic for visit 2 and were included in the study. The majority (548, 83.9%) were women who attended the clinic for family planning services (screening group). Another 105 women were referred because of an abnormal pap smear or for clinical reasons (referred group).

The distribution of demographic characteristics and risk factors for both groups is shown in Table 1. No significant differences in CIN detection or demographical variables were found between the group presenting for V1 and the group attending for V2 (data not shown).

3.2. Detection of CIN and carcinoma

A total of 255 biopsies were taken, on which 114 lesions were found. Table 2 shows the number of CIN lesions and invasive squamous cell carcinoma in the screening and the referred group. Lesions were more prevalent in the referred group. For assessment of the test qualities of the different screening methods, results from both groups are combined.

3.3. Pap smear

Of the 653 pap smears, 629 were satisfactory for evaluation (96.3%) (Table 3), and 16.7% of the adequate smears showed LSIL or worse. All 6 cases of invasive squamous cell cancer were detected. Of the 54 CIN2/3 cases, 44 (81.5%) were diagnosed on pap smear as LSIL or worse. Similarly, 27 (50.0%) of the 54 CIN1 cases were detected. Of the positive pap smears, 26.7% yielded a normal reference test.

3.4. VIA

One hundred seventy-seven VIA examinations were positive at visit 1 (27.1%) (Table 3). Four of the invasive squamous cell cancers were diagnosed on pap smear as LSIL or worse. Similarly, 27 (50.0%) of the 54 CIN1 cases were detected. Of the positive VIA tests, 26.7% yielded a normal reference test.

3.5. HPV DNA PCR

Five hundred sixteen HPV DNA PCR test results were available (Table 3). For hundred twenty
women, no HPV sample was taken, because of
unavailability at the clinic of the PBS sampling
medium, which had to be prepared freshly on
a regular basis. Four HPV tests had a border-
line (equivocal) result and 13 were indetermi-
nate. These cases were excluded from the
analysis.

Two hundred fifty-six (49.6%) women had a
positive HPV test, 193 (37.4%) were infected with
a HR HPV type. All invasive cancers were positive
for a HR HPV type. Forty-six (95.8%) of the CIN2/
3 cases had a positive HPV test, 45 (93.8%) had
an HR HPV type. Thirty-six (75.0%) of the women
with CIN1 were HPV positive, 34 (70.9%) were
infected with an HR HPV type. One hundred
sixty-eight (65.6%) women with HPV had a normal
reference test, compared to 108 (56.0%) of the
women with HR HPV.

3.6. Cervicography

A total of 523 cervicographies were taken in this
group (Table 3), and fifteen images were tech-
nically defective. One hundred and thirty cervi-
cographs indicated a cervical lesion. All the invasive
cancer cases tested positive, 29 (69.0%) of the CIN2/3
cases and 16 (33.3%) CIN1. Twenty-eight (35.9%) of the
women with a positive cervicography had a normal
reference test.

3.7. Comparison of the four screening tests

Table 4 shows estimates for the sensitivity and
specificity of the four screening tests. For cases of
CIN2 or worse, the pap smear showed a sensitivity of 83.3% and a specificity of 90.3%. The highest sensitivity (96.3%) was found with HPV testing (any type). However, the rate of false positives was considerably higher, yielding a specificity of 55.8%. Considering only HPV HR results as positive test results did not change the sensitivity significantly (94.4%), but did increase the specificity (69.3%). VIA scored a sensitivity and specificity of 73.3% and 77.6%, respectively, and cervicography 72.3% and 90.5%, respectively.

### 4. Discussion

This study was designed to compare the test qualities of alternative screening methods for the detection of CIN and invasive cervical cancer, which could be used in poor resource regions. This was a clinic-based population with a small proportion of referred women who contributed to the high number of (pre-)cancerous lesions.

Biopsy results were missing for 20 women with positive VIA2 but negative colposcopy, and 6 women with positive VIA2 and colposcopy suggestive of a low grade lesion. All 26 women had a negative pap smear. We kept these cases in the dataset, considering them negative on the reference test. Deleting these cases from the analysis would introduce a considerable bias towards fewer false positive VIA results.

The pap smear performed well with a sensitivity of 83.3% and a specificity of 90.3% for CIN2+. This test was performed at a referral and training center, which cautions that these results cannot be generalized for other cytology laboratories in the region. Indeed, a meta-analysis on pap test accuracy reported ranges for sensitivity and specificity of 11—99% and 14—97%, respectively [7]. This author concluded that pap test accuracy was not associated with reported study characteristics or dimensions of quality. In a similar study in Zimbabwe, pap smear performed with a sensitivity and specificity of 44.3% and 90.6%, respectively [8].

Most studies on HPV testing for cervical cancer screening use the Digene HPV Hybrid Capture II test, which detects 13 HR HPV types and 5 LR HPV types. Compared to our method, the Digene test had a slightly lower sensitivity and higher specificity in South Africa (88.4% and 81.9%, respectively) [9], in Costa Rica (88.4% and 89%) [10], in Zimbabwe (81% and 62%) [11], and in older women (35—45 years) in China (95% and 85%) [12]. The high cost and the low specificity of the test are important drawbacks. However, it has the potential
Comparison of pap smear, visual inspection

Comparison of pap smear, visual inspection

125

women over thirty years, as HPV infection is less prevalent but more often persistent in older women [13]. Visual screening techniques have been evaluated more intensely in the last decade. The two visual screening methods, VIA and cervicography, however, was significantly more specific. Our study adds to the VIA test characteristics found in other studies (sensitivity and specificity for detection of CIN2+ disease was 71.0% and 74.3%, respectively, in China [12], 76.6% and 64.1% in Zimbabwe [8], 82.6% and 86.5% in India [14], and 67.4% and 84.9% in South Africa [15]).

Two studies on cervicography provide sensitivity and specificity for CIN2+ disease of 54.5% and 97.2% [16], respectively, and 49.3% and 95.0% [6]. A study from South Africa reports sensitivity and specificity of 41.8% and 78.8%, respectively, for detection of any SIL [17]. Our study reports a significantly higher sensitivity with a comparable specificity compared to the studies mentioned above.

Colposcopy and colposcopy-directed biopsy to confirm a positive test result on the primary screening test is widespread, and is recommended policy in the industrialized world. This policy is cumbersome to implement in poor-resource countries. Colposcopy facilities are costly and require highly trained medical personnel. The prospect of having one test that is sensitive enough for screening, and at the same time specific enough to direct treatment is appealing. Inevitably, a number of false-positive cases would be treated unnecessarily, but a screen- and treat approach using cryotherapy, which has relatively few side-effects, has shown to be highly acceptable in poor resource settings [18]. In addition, excision or destruction of the transformation zone are considered preventive for later development of CIN. HIV-seropositive women, however, may shed the virus more intensely in the first weeks after treatment [19], increasing infectiousness in case of unprotected sexual intercourse. During the healing period, the cervix is also more fragile and more susceptible to HIV infection. However, it is also possible that, as the cervix heals after treatment and new epithelium emerges, often with a shrunken squamo-columnar junction, the seronegative women may actually get some long term protective effect from HIV acquisition. These aspects have to be considered carefully in regions with high HIV prevalence. Pap smear and cervicography have the highest specificity and would therefore cause the least over-treatment. HPV testing is the least specific and the specificity of VIA is intermediate but acceptable.

The lack of cervical cancer control programs in developing countries is largely due to the cost of those programs, which have to compete for resources with other health interventions. We did not assess cost-effectiveness of the different strategies, yet VIA was shown to be a cost-saving strategy in South Africa, compared to HPV HR testing and cytology [20].

A limitation of our study is that a number of HPV tests and cervicographies are missing or non-interpretable. As this is due to logistics and/or technical shortages, bias towards the overall results for those tests is unlikely. On the other hand, it also demonstrates the difficulties with using these screening methods in poor resource settings.

In conclusion, pap smear has the highest specificity, and HPV the highest sensitivity. Cervicography and VIA are comparable and score in between. However, from the perspective of the need for a simple and widely applicable screening test for cervical (pre-)cancer in poor resource countries, our study contributes to the growing evidence of the effectiveness of VIA as a primary screening tool.

Acknowledgments

The authors gratefully acknowledge the Family Planning Association of Kenya for their continued support to the study in availing their premises and highly motivated staff, without whom the study would not have been possible.

References

[4] Atlas of visual inspection of the cervix with acetic acid (VIA) [Chart]. JHPIEGO.


4. DISCUSSION

4.1. Contribution of this work to the field

East Africa has one of the highest burdens of cervical cancer worldwide. It is the leading cause of cancer mortality among women. The reason is basically twofold. Firstly, population-scale cervical cancer prevention through screening and treatment of pre-cancerous lesions is non-existent. Secondly, scarce data from the region has shown that the prevalence of HR HPV is very high, and ICC incidence is associated with HPV prevalence in a given population. This region has one of the highest prevalence rates in adult women with HIV and WHIV are more at risk for ICC.

In limited-resource regions, health policies are constantly challenged by the balance between health priorities, available human and technological resources and cost-effectiveness of health interventions. It is therefore crucial to improve our knowledge of screening technologies for poor-resource regions, as well as HPV epidemiology, in the context of cervical cancer screening by HPV testing, and implementation of the new prophylactic HPV vaccines.

We assessed test characteristics of alternative screening methods (Pap smear, VIA, HPV testing and cervicography) in a family planning clinic population in Nairobi. A visual training atlas was developed, which was used for training nurses in studies in Nicaragua [Claeys et al., 2003a] as well as Mombasa, Kenya. An assessment was also made of the efficiency of screening programmes in family planning clinics [Claeys et al., 2003b].

We performed an HPV prevalence survey among the FP population in Nairobi and conducted a case-control study, comparing HPV type distribution among women with ICC, both with and without HIV infection, who were admitted to the national referral hospital of Nairobi.

Using the lessons learnt from the Nairobi studies, we set up an operational research study on cervical screening in Mombasa, Kenya, covering the Mombasa District. Screening was integrated at the primary health care level and VIA was compared to Pap smear testing in field conditions. An age-stratified HPV survey was conducted among the screened population.

The results of the above described research will be discussed through the following topics:
1. HPV prevalence in Kenya: general population and cervical cancer
2. Impact of HIV on cervical cancer and HPV
3. Prevention of cervical cancer through alternative screening methods and HPV vaccination
4.2. HPV in Kenya

In the general population

We conducted HPV prevalence surveys among clients of family planning services as an approximation of the general population in Nairobi and Mombasa. For the Mombasa population, we applied an age stratification (100 women per 5 year age group from 15 to 55+) in the selection of the study group, similar to the IARC HPV Prevalence Surveys (IHPS).

HPV prevalences were amongst the highest found in worldwide populations to date: 44.3% (30.8% HR) in Nairobi and 44.7% (34.6% HR) in Mombasa. In Table 4.1, the prevalence of HPV types in HPV-positive samples from women with a normal cervix in Nairobi and Mombasa are compared with the worldwide distribution from the pooled IHPS analysis [Clifford et al., 2005a]. In this comparison, we found a 4-fold higher HPV prevalence in Nairobi and Mombasa. Where worldwide, HPV16 was two-fold more prevalent than the second most frequent HPV type (42), this was not the case for Nairobi or Mombasa, where HPV52 and HPV58 were about 50% more prevalent than HPV16. Other globally most frequently occurring HPV types (18, 31) were found in similar proportions at the two Kenyan sites.

The worldwide highly prevalent LR type HPV42 was not found in Nairobi, and was rare in Mombasa. Another LR type HPV53 was 6-fold and 13-fold more prevalent in Nairobi and Mombasa, respectively, compared to the worldwide distribution. HPV44 was significantly increased only in Nairobi, and not in Mombasa.

Although the two Kenyan studies used different HPV PCR assays, and it is known that sensitivities for specific types are slightly different per assay (the majority of the Mombasa samples were tested with the same assay as the IARC studies), these results again show the variability of HPV type prevalences across populations, even within the same country.
Table 4.1: Comparison of prevalence of HPV types in HPV-positive samples from women with normal cervix in Nairobi, Mombasa and worldwide*.

<table>
<thead>
<tr>
<th>Normal cervix % in HPV-positive samples</th>
<th>Nairobi N = 369</th>
<th>Mombasa N = 560</th>
<th>World-wide N = 15613</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% 95% CI</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
</tr>
<tr>
<td>Any HPV †</td>
<td>38.8 33.8 - 43.9</td>
<td>43.2 39.1 - 47.4</td>
<td>9.2 8.7 - 9.6</td>
</tr>
<tr>
<td>Any HR HPV</td>
<td>61.5 53.0 - 69.55</td>
<td>77.3 71.5 - 82.4</td>
<td>66.8 64.3 - 69.3</td>
</tr>
<tr>
<td>HPV 16 4.9 - 15.0</td>
<td>9.1 4.9 - 15.0</td>
<td>15.3 11.0 - 20.5</td>
<td>19.7 17.7 - 21.9</td>
</tr>
<tr>
<td>HPV 18 2.4 - 10.7</td>
<td>5.6 2.4 - 10.7</td>
<td>9.9 6.5 - 14.4</td>
<td>7.2 5.9 - 8.7</td>
</tr>
<tr>
<td>HPV 31 4.4 - 14.2</td>
<td>8.4 4.4 - 14.2</td>
<td>9.1 5.8 - 13.4</td>
<td>7.5 5.9 - 8.7</td>
</tr>
<tr>
<td>HPV 33 2.0 - 9.8</td>
<td>4.9 2.0 - 9.8</td>
<td>7.0 4.1 - 11.0</td>
<td>-</td>
</tr>
<tr>
<td>HPV 35 3.4 - 12.5</td>
<td>7.0 3.4 - 12.5</td>
<td>6.6 3.8 - 10.5</td>
<td>5.9 -</td>
</tr>
<tr>
<td>HPV 39 1.1 - 8.0</td>
<td>3.5 1.1 - 8.0</td>
<td>4.1 2.0 - 7.5</td>
<td>4.3 -</td>
</tr>
<tr>
<td>HPV 42 0.0</td>
<td>0.0 -</td>
<td>2.1 0.7 - 4.8</td>
<td>9.4 -</td>
</tr>
<tr>
<td>HPV 45 1.6 - 8.9</td>
<td>4.2 1.6 - 8.9</td>
<td>3.7 1.7 - 6.9</td>
<td>5.6 -</td>
</tr>
<tr>
<td>HPV 52 10.5 - 23.1</td>
<td>16.1 10.5 - 23.1</td>
<td>12.4 8.5 - 17.2</td>
<td>4.7 -</td>
</tr>
<tr>
<td>HPV 56 1.1 - 8.0</td>
<td>3.5 1.1 - 8.0</td>
<td>5.4 2.9 - 9.0</td>
<td>7.1 -</td>
</tr>
<tr>
<td>HPV 58 3.4 - 12.5</td>
<td>7.0 3.4 - 12.5</td>
<td>22.3 17.2 - 28.1</td>
<td>5.3 -</td>
</tr>
<tr>
<td>HPV 66 4.9 - 15.0</td>
<td>9.1 4.9 - 15.0</td>
<td>7.4 4.5 - 11.5</td>
<td>4.1 -</td>
</tr>
<tr>
<td>HPV 68 1.6 - 8.9</td>
<td>4.2 1.6 - 8.9</td>
<td>5.8 3.2 - 9.5</td>
<td>2.1 -</td>
</tr>
<tr>
<td>LR HPV ‡</td>
<td>18.9 12.8 - 26.3</td>
<td>- ¥  -</td>
<td>27.7 -</td>
</tr>
<tr>
<td>HPV 6 0.2 - 5.0</td>
<td>1.4 0.2 - 5.0</td>
<td>10.3 6.8 - 14.9</td>
<td>1.4 -</td>
</tr>
<tr>
<td>HPV 11 0.2 - 5.0</td>
<td>1.4 0.2 - 5.0</td>
<td>5.8 3.2 - 9.5</td>
<td>1.4 -</td>
</tr>
<tr>
<td>HPV 44 2.9 - 11.6</td>
<td>6.3 2.9 - 11.6</td>
<td>1.2 0.3 - 3.6</td>
<td>0.7 -</td>
</tr>
<tr>
<td>HPV 53 3.4 - 12.5</td>
<td>7.0 3.4 - 12.5</td>
<td>15.3 11.0 - 20.5</td>
<td>1.2 -</td>
</tr>
<tr>
<td>HPV X 13.4 - 27.0</td>
<td>19.6 13.4 - 27.0</td>
<td>2.9 1.2 - 5.9</td>
<td>5.5 -</td>
</tr>
</tbody>
</table>

† Percentage of HPV positive tests in the total sampled population
‡ Samples with exclusively LR HPV types
¥ No data available for exclusively LR HPV types
± For types other than 16 and 18, it is not possible to calculate 95% confidence intervals, as the total number of tested samples is not mentioned in the publication

These results are relevant when estimating the test characteristics in the use of HR HPV screening to detect women at high risk for HSIL or cancer in this region. In Mombasa, we found a very high HR HPV prevalence among normal women and a slightly increased prevalence in
women with an abnormal cervix. This means that HR HPV testing will have a limited power to discriminate normal women from women with abnormalities. We estimated test characteristics for the commercially available HC2 test, applied to the Mombasa study population. Of the 618 women tested, 214 had a positive HC2 test, and 27 of these had an abnormal cervix (ASCUS or more). Thirty-one women with cervical lesions would be missed. The calculation yields a sensitivity of 46.6%, specificity of 66.6%, positive predictive value of 12.6% and negative predictive value of 92.3%.

**In invasive cervical carcinoma**

Our study contributes to the growing knowledge on HPV type distribution in ICC in Africa. To date, studies have reported on Algeria, Benin, Ethiopia, Guinea, Mali, Morocco, Mozambique, Senegal, South Africa, Tanzania, Uganda and Zimbabwe [Smith et al., 2007]. We compared HPV types among Kenyan HIV-negative women with ICC to the worldwide distribution (Table 4.3). We demonstrated a lower prevalence of HPV16 and higher prevalence of HPV35, HPV45 and HPV52. Previous reports have shown higher prevalences of HPV35, HPV45 and HPV58 in women from sub-Saharan Africa, compared to North America and Europe [Clifford and Franceschi, 2005]. Overall, HPV16 and/or HPV18 prevalence was 62% in Nairobi, which is at the lower end of the range found in the world-wide meta-analysis, notably 65% in South/Central America and 76% in North America.
Table 4.2. Comparison of prevalence of HR type-specific HPV infection among cervical carcinoma cases by HIV status, Nairobi, Kenya to worldwide distribution of HR HPV types.

<table>
<thead>
<tr>
<th></th>
<th>HIV+ N = 51 %</th>
<th>95% CI</th>
<th>HIV- N = 153 %</th>
<th>95% CI</th>
<th>Worldwide N = 14595 %</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV positive</td>
<td>100.0</td>
<td>93.0 - 100.0</td>
<td>96.7</td>
<td>92.5 - 98.9</td>
<td>86.9</td>
<td>86.3 - 87.4</td>
</tr>
<tr>
<td>Any multiple</td>
<td>37.3</td>
<td>24.1 - 51.9</td>
<td>13.7</td>
<td>8.7 - 20.2</td>
<td>7.8</td>
<td>7.4 - 8.2</td>
</tr>
<tr>
<td>HPV 16</td>
<td>41.2</td>
<td>27.6 - 55.8</td>
<td>43.8</td>
<td>35.8 - 52.0</td>
<td>55.2</td>
<td>54.2 - 56.2</td>
</tr>
<tr>
<td>HPV 18</td>
<td>27.5</td>
<td>15.9 - 41.7</td>
<td>17.6</td>
<td>12.0 - 24.6</td>
<td>12.8</td>
<td>12.1 - 13.5</td>
</tr>
<tr>
<td>HPV 16/18</td>
<td>64.7</td>
<td>50.1 - 77.6</td>
<td>60.1</td>
<td>51.9 - 67.9</td>
<td>70.3</td>
<td>69.5 - 71.0</td>
</tr>
<tr>
<td>HPV 31</td>
<td>5.9</td>
<td>1.2 - 16.2</td>
<td>3.3</td>
<td>1.1 - 7.5</td>
<td>3.8</td>
<td>- ±</td>
</tr>
<tr>
<td>HPV 33</td>
<td>5.9</td>
<td>1.2 - 16.2</td>
<td>2.6</td>
<td>0.7 - 6.6</td>
<td>3.7</td>
<td>-</td>
</tr>
<tr>
<td>HPV 35</td>
<td>7.8</td>
<td>2.2 - 18.9</td>
<td>5.2</td>
<td>2.3 - 10.0</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>HPV 39</td>
<td>0.0</td>
<td>-</td>
<td>3.3</td>
<td>1.1 - 7.5</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>HPV 45</td>
<td>7.8</td>
<td>2.2 - 18.9</td>
<td>17.0</td>
<td>11.4 - 23.9</td>
<td>4.6</td>
<td>-</td>
</tr>
<tr>
<td>HPV 51</td>
<td>3.9</td>
<td>0.5 - 13.5</td>
<td>2.0</td>
<td>0.4 - 5.6</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>HPV 52</td>
<td>19.6</td>
<td>9.8 - 33.1</td>
<td>5.2</td>
<td>2.3 - 10.0</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>HPV 56</td>
<td>5.6</td>
<td>1.2 - 16.2</td>
<td>0.7</td>
<td>0.02 - 3.6</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>HPV 58</td>
<td>0.0</td>
<td>-</td>
<td>1.3</td>
<td>0.16 - 4.6</td>
<td>2.8</td>
<td>-</td>
</tr>
<tr>
<td>HPV 59</td>
<td>0.0</td>
<td>-</td>
<td>0.7</td>
<td>0.02 - 3.6</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>HPV 66</td>
<td>3.9</td>
<td>0.5 - 13.5</td>
<td>0.7</td>
<td>0.02 - 3.6</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>HPV 68</td>
<td>3.9</td>
<td>0.5 - 13.5</td>
<td>2.6</td>
<td>0.7 - 6.6</td>
<td>0.5</td>
<td>-</td>
</tr>
</tbody>
</table>


± For types other than 16 and 18, it is not possible to calculate 95% confidence intervals, as the total number of tested samples is not mentioned in the publication.
Lesion-specific HPV type distributions in women in Nairobi

We combined the HPV type distributions found in the two studies from Nairobi among women attending for family planning and women with ICC (Table 4.2 and Figure 4.1). As HIV prevalence was low in the family planning population (11.5%), we choose to compare HPV results with those of the HIV-negative women with ICC. In both studies, HPV testing was done on cervical cells on swabs, with the same technology (SPF10_INNO/LiPA) and processed at the same laboratory. We therefore believe the HPV results from both studies are comparable.

Not surprisingly, we see a gradual ‘enrichment’ of the proportion of HPV16 among HPV positive samples with increasing severity of the lesions. For the phylogenetically linked HPV18 and 45, we do not report a noticeable increase in pre-cancerous lesions, but a marked increase in ICC, represented the second and third most important HPV type (18,2 and 17.6% of samples, respectively). Types 35 and 52 are highly prevalent in normal and pre-cancerous women, but lose importance in samples from women with ICC (5.2% of samples for both). Moreover, both HPV35 and HPV52 are very frequently found in multiple type infections in normal and pre-cancerous lesions (13/22 and 20/34, respectively). In HIV-negative women with ICC, HPV35 and HPV52 are found as multiple type infections in 6/8 and 2/8 of occurrences, respectively. Although it is not precisely known how individual oncogenicity within multiple type infections should be interpreted, this fact further downplays the importance of HPV52 in particular in HIV-negative ICC.

We reported a relatively high prevalence of the globally rarely reported HPV66 in normal and pre-cancerous lesions (9.1 – 10.7%), and we considered its oncogenic potential within our family planning population. However, HPV66 was found in only 0.7% in ICC, which is in accordance with its classification as “possibly high-risk” [Munoz 2003].
Table 4.3. Comparison of distribution of HPV types in HPV positive samples from women with normal cervix, pre-cancer and cancer in Nairobi, Kenya.

<table>
<thead>
<tr>
<th></th>
<th>Normal N=369</th>
<th>LSIL N=30</th>
<th>HSIL N=29</th>
<th>Inv Ca† N=153</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>HPV pos. %</td>
<td>HPV pos. %</td>
<td>HPV pos. %</td>
<td>HPV pos. %</td>
</tr>
<tr>
<td>HPV Neg</td>
<td>226</td>
<td>12</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>HPV pos</td>
<td>143</td>
<td>100.0</td>
<td>18</td>
<td>100.0</td>
</tr>
<tr>
<td>HPV HR</td>
<td>27</td>
<td>18.9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>HPV X</td>
<td>88</td>
<td>61.5</td>
<td>16</td>
<td>88.9</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>HPV pos. %</td>
<td>HPV pos. %</td>
<td>HPV pos. %</td>
<td>HPV pos. %</td>
</tr>
<tr>
<td>HPV LR</td>
<td>6</td>
<td>2</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>HPV HR</td>
<td>11</td>
<td>2</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td>HPV X</td>
<td>43</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>HPV LR</td>
<td>44</td>
<td>9</td>
<td>6.3</td>
<td>1</td>
</tr>
<tr>
<td>HPV HR</td>
<td>53</td>
<td>10</td>
<td>7.0</td>
<td>3</td>
</tr>
<tr>
<td>HPV X</td>
<td>54</td>
<td>8</td>
<td>5.6</td>
<td>2</td>
</tr>
<tr>
<td>HPV LR</td>
<td>70</td>
<td>6</td>
<td>4.2</td>
<td>1</td>
</tr>
<tr>
<td>HPV HR</td>
<td>74</td>
<td>7</td>
<td>4.9</td>
<td>1</td>
</tr>
<tr>
<td><strong>16</strong></td>
<td>13</td>
<td>9.1</td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td><strong>18</strong></td>
<td>8</td>
<td>5.6</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td><strong>31</strong></td>
<td>12</td>
<td>8.4</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>33</strong></td>
<td>7</td>
<td>4.9</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>35</strong></td>
<td>10</td>
<td>7.0</td>
<td>7</td>
<td>38.9</td>
</tr>
<tr>
<td><strong>39</strong></td>
<td>5</td>
<td>3.5</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>40</strong></td>
<td>1</td>
<td>0.7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>45</strong></td>
<td>6</td>
<td>4.2</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>51</strong></td>
<td>4</td>
<td>2.8</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>52</strong></td>
<td>23</td>
<td>16.1</td>
<td>4</td>
<td>22.2</td>
</tr>
<tr>
<td><strong>56</strong></td>
<td>5</td>
<td>3.5</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>58</strong></td>
<td>10</td>
<td>7.0</td>
<td>2</td>
<td>11.1</td>
</tr>
<tr>
<td><strong>59</strong></td>
<td>1</td>
<td>0.7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>66</strong></td>
<td>13</td>
<td>9.1</td>
<td>1</td>
<td>5.6</td>
</tr>
<tr>
<td><strong>68</strong></td>
<td>6</td>
<td>4.2</td>
<td>1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

† Among HIV seronegative women
‡ Samples with exclusively LR HPV types
Figure 4.1: HPV type distribution of the most frequent types as a proportion of HPV positive cases in cervical lesions of increasing severity in Nairobi, Kenya.
South- and East-Africa have been hit hard by the HIV epidemic. Several epidemiological studies have therefore tried to elucidate the effect of the HIV epidemic on the incidence of ICC in populations with high HIV prevalence. As described in the introduction section, cancer registry studies from Uganda [Parkin et al., 1999] and Zimbabwe [Chokunonga et al., 1999] could not show a temporal increase in ICC incidence over time periods that HIV prevalence dramatically increased in the population, in contrast to other HIV associated cancers. Our study reviewed patient records from the national referral hospital in Nairobi and could also not show an increase in presentation of ICC at the hospital over the period 1989 to 1998, during which time the national HIV prevalence rate increased from 5% to 15%. HIV-positive women were on average 5 years younger than HIV-negative women. Only recently has a cancer record-linkage study been able to show an increased risk of ICC in women with HIV [Mbulatseye et al., 2006].

Subsequently, in our prospective case-control study in the same hospital in women with ICC and controls with uterine fibroids, we found that among women younger than 35 years, women with ICC were 2.6 times more likely to be HIV-positive than controls. WHIV with ICC were also 10 years younger on average than HIV-negative women with ICC. Most women were diagnosed at later cancer stages for HIV-positive and –negative women alike, however, WHIV were three times more likely to have histologically poorly differentiated tumours than women without HIV, suggesting a worse prognosis in WHIV.

Not surprisingly, HIV-positive women in the Nairobi FP population were 87% HPV-positive, compared to 39% of HIV-negative women. Conversely, among women with HR HPV, only 6% of HIV-positive women had a HSIL lesion, compared to 27% of HIV-negative women. This suggests a weaker association between HR HPV and SIL in HIV-positive women, compared to HIV-negative women. This contradictory finding was reported earlier from Nairobi, in commercial sex workers [Kreiss et al., 1992], as well as in a comparable family planning population [Temmerman et al., 1999].

As previously reported in women with normal or pre-cancerous cervical findings, WHIV and ICC similarly had an increased risk of multiple HPV infection [Clifford et al., 2006b]. The underrepresentation of HPV-16, as shown in WHIV with normal or pre-cancerous cervical findings was not seen in our study among women with ICC. The only types with an overall borderline statistically different distribution in HIV-positive women were HPV-45 (less
prevalent) and HPV-52 (more prevalent). The latter association has been reported previously in
WHIV with high grade cervical squamous intraepithelial lesions (HSIL) [Clifford et al., 2006b].
Our anecdotal HPV-66 was seen to be more prevalent in HIV positive samples, 3.9%,
compared to 0.7% in HIV-negative samples, however, all occurrences in HIV-positive samples
were in multiple type infections (data not shown), and the difference was not statistically
significant. In conclusion, we found a similar range of HPV types in HIV-positive, compared to
HIV-negative women across the range of lesions. It is relevant for HPV vaccination that the
prevalence of HPV16/18 was similar in both groups.
As pointed out in the results section, we unfortunately have no information on age at HIV
infection. We cannot, therefore, rule out the possibility that HPV infection, which is very
common in sub-Saharan Africa and steeply increases after sexual debut, had been acquired
years before the HIV seroconversion. In Kenya, the HIV epidemic experienced its greatest
prevalence increase in the mid nineties and half of the WHIV in our study group had normal
immunity, which suggests rather recent HIV seroconversion. This might possibly bias our
present comparison of HIV-positive and HIV-negative women towards lack of significant
difference, as HIV infection is unlikely to affect HPV type after the HPV-induced cancer
process has become irreversible.

4.3. Prevention of cervical cancer in Kenya

4.3.1. Prevention of cervical cancer through alternative screening methods:

Cytology:
Although Pap smear performed with a good sensitivity and specificity our Nairobi study, there
is growing evidence that the test is not very suitable for resource-poor settings, mainly for
quality reasons resulting in a low sensitivity. We also found this in the screening programme in
Nicaragua. Therefore, screening programmes should either carefully maintain the quality of the
Pap smear technique, or should choose to use alternative screening methods. One way of
improving the quality of this test is to centralise the Pap smear lab, in order to control the
quality and to try to decrease the load by applying a triage test to exclude women who are at
reduced risk of SIL. Although insufficiently studied in limited-resource settings, the use of
liquid-based cytology might also improve the performance of cytological testing, mainly through increasing the proportion of slides that are satisfactory for assessment. It is however very unlikely that this method would be realistic, as it is costly, increases the level of technology required. A prospective randomized study from Switzerland demonstrated that simply removing mucus and cellular debris from the cervical surface with a cellulose swab before sampling cells resulted in the same specimen adequacy for conventional cytology, compared to LBC [Obwegeser et al., 2001].

Overall, the high specificity of the Pap smear test remains an advantage that many alternative tests cannot offer and we showed that it is possible to provide a high quality Pap smear in a specialised setting in a developing country.

**Visual Inspection with Acetic Acid**

Visual screening techniques have been evaluated more intensively since the last decade, as one of the most promising alternative screening methods, because they are low cost and require no technological back-up. Our results confirm previous studies showing a high sensitivity, but relatively low specificity. The same was seen in the Nicaragua screening study, where compared to Pap smear, VIA was twice as sensitive in detecting CIN2 or worse, but for every extra detected case, 8 “false positive” cases were identified. This high rate of false-positive test results means that a confirmatory test is needed, which is basically colposcopy-directed biopsy. Alternatively, in a direct “see-and-treat” approach, a lot of false-positive healthy women would be treated. This could be acceptable if the treatment has no intrinsic morbidity, as was documented for cryotherapy in a “see and treat” approach in Thailand [Gaffikin et al., 2003].

Another randomized study from South Africa compared a see and treat approach, screening with VIA and HR-HPV testing in 4390 women to a group of 2165 women that were screened with a treatment delay of 6 months [Denny et al., 2005]. This approach was effective, as the prevalence of CIN2+ at 6 months post-treatment was significantly lower in the immediately treated group, compared to the delayed treatment group. This approach was also found safe as only one severe complication was noted in one HIV-seropositive woman who developed serious cervical bleeding, 2 weeks after treatment, requiring hospitalization. There was also no difference in HIV-seroconversion at 6 months post-treatment between the immediately treated group, and the women with delayed treatment. Moreover, ablation of the transformation zone through freezing is considered preventive for the development of SIL, and would therefore be beneficial to the false-positive healthy women. This has lead some groups such as PATH and IARC to support the see-and-treat approach now, albeit still cautiously. In any event, the burden
of over-treatment in already challenged health systems in poor resource countries still needs to be carefully studied.

Testing for HPV

HPV testing, using clinician- or self-collected vaginal swabs, has also been proposed as an adjunctive or alternative cervical cancer control measure, especially in developing countries [Ogilvie et al., 2005]. As stated above, PPV is an important test characteristic and should be as high as possible. The PPV will depend on the prevalence of HR-HPV in the screened population, as well as with the associations with CIN2+ (associated with persistent infection). Therefore, the efficiency of this test will be determined by the age-specific HR-HPV distribution in the population. Where HR-HPV testing as a primary screening test has been proposed for women older than 30 – 35 years in populations with peaking HPV incidences before the age of 25, it might have to be reconsidered for populations with similar high HPV prevalences in higher age ranges, as we showed for the Kenyan population.

We should also consider the fact that we found a weaker association between detection of HR HPV and SIL in women with HIV as this would mean that the test will have a lower specificity when used in populations with high HIV prevalence.

The cost of the test is currently an impediment to widespread use in limited-resource countries, but simple, low cost and fast tests are being developed for use in these countries through the PATH START project.

4.3.2. Prevention of cervical cancer through HPV vaccination:

In our Nairobi population, we found a lower proportion of HSIL associated with HPV16 or –18 (38%), compared to the worldwide distribution (52%) [Smith et al., 2007], which would suggest a lower protection against HSIL in the East-African population.

However, the ultimate goal of HPV vaccination is to prevent ICC. Our data show a similar prevalence of HPV16 and/or –18 in HIV-positive (64.7%) and HIV-negative (60.1%) women with ICC, which is at the lower end of the range found worldwide.

It is of some interest that we found a significantly higher prevalence of multiple type infections in WHIV with ICC (37.2%), compared to women with ICC without HIV (13.7%). Although the relevance of mixed type infections including HPV16 or 18 is still not clear, as is the protective
effect of the HPV vaccine in those cases, we considered a hypothetical scenario where the vaccine was only effective in cases with HPV16 and 18 as single type infection, or combined, or combined with a LR HPV type. This would have been the case for 19/51 (37.3%) of HIV-positive women, compared to 82/153 (53.6%) of HIV-negative women. This difference is remarkable, but statistically insignificant in our study (P=.3).

Overall, this is good news for the populations in Africa, including WHIV, although the aim should be to vaccinate girls before they become sexually active, and hence negative for HIV as well as HPV. As both viruses are transmitted through sexual contact, it is unlikely that a woman with HIV would be naive for HPV.

Given the high cost of the vaccine, special consideration will have to be given to methods of distribution in developing countries. As these populations have little access to health care by definition, this will be a particularly challenging aspect of vaccination programs, as is the administration of three injections to young adolescents. Hopefully, if long duration of the efficacy of HPV vaccine can be demonstrated, it should become possible to schedule HPV vaccines in childhood.

There are high expectations for HPV vaccination to be more efficient in preventing cervical cancer in Africa, compared to the failing screening programmes. However, it should be kept in mind that any benefits from vaccination campaigns can only be expected 20 – 30 years later. In the mean-time, screening programmes will have to be strengthened, especially for WHIV, who may become the highest risk group for cervical cancer, now that they are surviving longer thanks to antiretroviral treatment.

4.4. Conclusions

Our studies are the first to report the prevalence of HPV types in an East-African population. HPV prevalence is among the highest reported worldwide, with an underrepresentation of HPV16 and overrepresentation of HPV 52 and 58. Knowledge of regional HPV type distribution in the population is not only important in the study of the natural history of HPV, but also in the context of the possible design of region-specific HPV screening tests.

We are also the first to report on HPV prevalence in a substantial sample of women with cervical cancer in East-Africa. We found HPV type distributions in cervical cancer that were similar to worldwide distribution, despite the fact that a different distribution was found in the
general population. The same predominance was found for HPV16 and HPV18, thus confirming the fact that these HPV types are very oncogenic and their prevalence increases as the severity of cervical lesions worsens [Franceschi and Clifford, 2005]. This also means that the prophylactic HPV vaccines would offer an overall similar level of lower protection to elsewhere.

We also compared HPV type distribution in ICC in HIV-positive to HIV-negative women in by far the largest study sample reported. With the caveat of having studied women with relatively recent HIV infection, we found similar HPV type distributions, including HPV 16/18 prevalence in both groups, although multiple-type infections were more prevalent in WHIV. If our results are confirmed in larger studies, this would be good news for the protection of future generations of WHIV by HPV 16/18 vaccines.

However, before the first benefits of protection by the vaccines can be expected in 20 – 30 years time, there will be a need for cost-effective cervical cancer screening programmes in developing countries in which alternative screening methods will have their role to play. We found that VIA might be a particularly interesting method to explore further in this context.
4.5. Recommendations

As a result of our findings, we formulated the following recommendations:

1) Further research is needed on the importance of multiple type infections in the carcinogenic process, especially for those containing HPV16 or -18, and on the impact of vaccination against HPV16 or -18 in these instances.

2) Careful monitoring of HPV-related cancers and intensified prevention programmes will be needed in those developing countries with high HIV prevalence and increased accessibility to ART.

3) The safety and efficacy of HPV vaccines in people with HIV still needs to be assessed.

4) The issue of appropriate cervical cancer screening and treatment algorithms for poor resource countries still needs further exploration, especially through demonstration projects in field conditions.
5. EXECUTIVE SUMMARY

5.1. Context and Objectives

Background
In 2002, nearly 600,000 new cancers were attributed to human papillomavirus (HPV) infection (i.e., 5.2% of all cancer) worldwide (Parkin et al., 2006). Invasive cervical carcinoma (ICC) was the most common, 80% of which occurs in developing countries with the highest incidences found in sub-Saharan Africa. The vast majority of ICC is considered to be caused by oncogenic (high risk) human papillomavirus (HR HPV), most importantly types 16 and 18, which are associated with around 70% of cervical cancers. Other HR HPV types have oncogenic potential but to a lesser degree than HPV16 and 18. Despite the predominant role of HPV, other cofactors also play a role in the onset of HPV-related cancers, one of which is HIV-related immunosuppression.

The most important recent development in the field of HPV is the availability of prophylactic vaccines against infection with HPV16 and -18. At the same time, anti-retroviral treatment (ART) is becoming accessible to increasing proportions of HIV-infected populations in developing countries, most notably sub-Saharan Africa. Improved survival of people with HIV will, however, also increase the burden of HPV-related cancers.

Objectives
The general objective of this work is to describe the interaction between HPV and HIV, the epidemiology of human papillomavirus in Kenyan populations and the prevalence of HPV types and the impact of HIV in invasive cervical cancers in Kenya.

We specifically aimed 1) to describe the interaction between HIV, HPV and cervical dysplasia and cancer in the era of HAART; 2) to describe the prevalence of HPV types in a family planning population in Nairobi; 3) to describe the age-specific prevalence of HPV types in a family planning population in Mombasa; 4) to describe the prevalence of HPV types in invasive cervical cancer in Nairobi and the impact of HIV infection on HPV type distribution in these women; and 5) to compare test characteristics of several alternative screening methods for detection of cervical pre-cancer and cancer in poor-resource settings;
**Methods**

Data were obtained through literature review and studies in Nairobi and Mombasa, Kenya. This was done in collaborative studies with the Ghent University, Antwerp University, University of Nairobi, Ministry of Health, Kenya and the International Agency for Research on Cancer, Lyon (WHO).

In Nairobi, a study was conducted in a family planning clinic population, where an HPV survey was conducted and test characteristics of alternative screening methodologies were compared. In order to describe HPV type distribution in women with ICC, according to HIV status, a comparative case-case study (women with ICC both with and without HIV infection) was carried out in the national referral hospital in Nairobi.

In Mombasa, a population survey was conducted, assessing age-specific prevalence of HPV types in a family planning population older than 15 years and using an age-stratified subject selection (100 subjects per 5-year age group), in order to compare these data with the IARC HPV Population Surveys (IHPS).

In all studies, HPV testing was performed on cervical scrapes using PCR technology.

**5.2. Results**

**5.2.1. HPV in Kenya**

*Among the general population*

HPV prevalence surveys were conducted among family planning clinic clients as an approximation for the general population. HPV prevalences were amongst the highest found in populations worldwide to date: 44.3% (30.8% HR) in Nairobi and 44.7% (34.6% HR) in Mombasa. In Nairobi, among 369 women with normal cervix, 38.8% (95% CI 33.8 - 43.9) were HPV positive. The most common HPV types were HPV52 (16.1% (10.5 – 23.1) of positive samples), HPV16 and HPV66 (both 9.1% (4.9 – 15.0)) and HPV 31 (8.4% (4.4 – 14.2)). In Mombasa, among 560 women with normal cervix, 43.2% (95% CI 39.1 – 47.4) were HPV positive. The most common HPV types were HPV58 (22.3% (17.2 – 28.1) of positive samples), HPV16 (15.3% (11.0 – 20.5), HPV52 (12.4% (8.5 – 17.2) HPV18 (9.9% (6.5 – 14.4)) and HPV 31 (9.1% (5.8 – 13.4)).

Prevalence of HPV types in HPV-positive samples from women with normal cervix were compared for Nairobi and Mombasa with the worldwide distribution from a pooled IHPS
analysis [Clifford 2005]. In contrast to worldwide figures, where HPV16 was two-fold more prevalent than the second most frequent HPV type (42), this was not the case for Nairobi or Mombasa, where HPV52 and HPV58 were about 50% more prevalent than HPV16. Other globally most frequent HPV types (18, 31) were found in similar proportions in the two Kenya sites. These results again show the variability of HPV type prevalences across populations, even within the same country.

These results are relevant for estimating the test characteristics in the use of HR HPV screening to detect women at high risk for HSIL or cancer in this region. In Mombasa, we found a very high HR HPV prevalence among normal women (33.4%) with a slightly increased prevalence in women with an abnormal cervix (46.6%). This means that HR HPV testing will have a limited power to discriminate normal women from women with abnormalities with a low positive predictive value (12.6% for Mombasa).

In invasive cervical carcinoma

Our study contributes to the growing knowledge on HPV type distribution in ICC in Africa. In Nairobi, among 153 HIV-negative women with ICC, we found a high prevalence of multiple type infections (13.7% (95% CI 8.7 – 20.2)). The most common HPV types were HPV16 (43.8% (35.8 – 52.0)), HPV18 (17.6 (12.0 – 24.6), HPV45 (17.0 (11.4 – 23.9) and HPV35 and HPV52 (both 5.2% (2.3 – 10.0)). Compared to worldwide (WW) distribution of the above mentioned types in ICC (Smith et al, 2007), we showed a lower prevalence of HPV16 (55.2% (54.2 – 56.2) WW), higher prevalence of HPV35 (1.5% (1.2 – 1.8) WW), HPV45 (4.6 (4.1 – 5.2) and HPV52 (2.9 (2.5 – 3.3) WW). Higher prevalences of HPV35, HPV45 and HPV58 were previously noted in sub-Saharan Africa, compared to North America and Europe [Clifford et al. 2005].

Comparing the HPV type distributions from normal to pre-cancerous and cancerous (HIV-negative) lesions among study participants in the two Nairobi studies, we see a gradual “enrichment” of HPV16. HPV 18 and 45 were notably prevalent in ICC (both about 18% of positive samples). Conversely, the highly prevalent HPV types 35 and 52 in normal and precancerous cervix were much less frequent in ICC (both 5.2%).

Impact of HIV

A review of patient records of women with ICC admitted at the national referral hospital in Nairobi between 1989 and 1998 revealed no increased incidence of ICC, while the background HIV prevalence in the population tripled from 5% to 15% over the same period. In a subsequent
case-control study in the same hospital among women with ICC and controls with fibroids, we demonstrated that only among women younger than 35 years, women with ICC were 2.6 times more likely to be HIV-positive than among women with fibroids. WHIV with ICC were also on average 10 years younger than women with ICC without HIV.

Not surprisingly, among the HIV-positive women in the Nairobi FP population, 87% were HPV-positive, compared to 39% among HIV-negative women. Conversely, among women with HR HPV, only 6% of HIV-positive women had a HSIL lesion, compared to 27% among HIV-negative women. This suggests a weaker association between HR HPV and SIL in HIV-positive women, compared to HIV-negative women. The same finding was reported earlier in Nairobi, in commercial sex workers [Kreiss et al., 1992], as well as in a comparable family planning clinic population [Temmerman et al., 1999].

Comparing HPV type distribution in ICC in HIV-positive women to HIV-negative women, the most striking finding was the increased risk of multiple HPV infection in HIV-positive women (37.2% vs. 13.7%, respectively). Similar findings were previously reported in women with normal or pre-cancerous cervical findings for HIV-positive, compared to HIV-negative women [Clifford et al., 2006b]. The underrepresentation of HPV16, as shown in the latter group was not seen in our study among women with HIV with ICC. The only types with an overall borderline statistically different distribution in HIV-positive women was HPV-45 (less prevalent) and HPV-52 (more prevalent). The latter association was already reported previously in WHIV with high grade cervical squamous intraepithelial lesions (HSIL) [Clifford et al., 2006b]. We found a similar range of HPV types in HIV-positive, compared to HIV-negative women. It is relevant for HPV vaccination, that the prevalence of HPV16/18 was similar in both groups.

Half of the WHIV in our study group had normal immunity (CD4+ > 500), which suggests rather recent HIV seroconversion. This would possibly bias our present comparison of HIV-positive and HIV-negative women towards lack of difference, as HIV infection is unlikely to affect HPV type after the HPV-induced cancer process has become irreversible.

5.2.2. Prevention of cervical cancer in Kenya through HPV vaccination

Although we found a lower proportion of HSIL associated with HPV16 or –18 (38%), compared to the worldwide distribution (52%)[Smith et al., 2007], which would suggest a lower protection against HSIL in the East-African population, our data from women with ICC show a similar prevalence of HPV16 and/or –18 in HIV-positive (64.7%) and HIV-negative (60.1%)
women, which is at the lower end of the range found worldwide. This is good news for the populations in Africa in general, as well as for women with HIV, although the aim should be to vaccinate girls before they become sexually active, and hence negative for HIV as well as HPV. The high cost of the vaccine, as well as vaccination of young adolescents will pose specific challenges in developing countries.

5.2.3. Prevention of cervical cancer in Kenya through alternative cervical screening methodologies

The test performance of visual inspection with acetic acid (VIA) was first looked at in the cross-sectional study among 653 family planning attenders in Nairobi, Kenya. To detect a threshold of HSIL or worse disease, Pap smears were shown to have a sensitivity and a specificity of 83.3% (95% CI 71.5-91.7) and 94.6% (95% CI 92.3-96.4) respectively. For VIA this was 73.3% (95% CI 84.6-98.8) and 80.0% (95% CI 76.3-83.3); for high-risk HPV 94.4% (95% CI 84.6-98.8) and 73.9% (95% CI 69.4-78.1); and for cervicography 74.5% (95% CI 59.7-86.1) and 89.9% ((95% CI 86.5-92.6). The Pap test had an excellent performance in this research setting, using a highly qualified nurse for the examination and a cytology training facility for the reading of the test. The low-cost, low-tech VIA method also performed adequately.

The performance of VIA in field conditions as a screening test for cervical (pre-) cancer was assessed in Rivas, Nicaragua among 1,076 women, concurrently screened with VIA and Pap smear [Claeys et al., 2003a]. Four percent were positive on both VIA and Pap, 25% on VIA only and 3% on Pap smear only. Twice as many CIN2-3 and ICC were detected through VIA compared to the Pap smear. However, for every extra detected case, 8 “false positive” cases were identified, which also needed referral for confirmation. This is an important aspect to consider in poor-resource settings with usually already over-burdened referral levels.

5.3. Conclusions and recommendations

Our studies are the first to report the prevalence of HPV types in an East-African population. HPV prevalence is among the highest reported worldwide, with an underrepresentation of
HPV16 and overrepresentation of HPV 52 and 58. This information is important in the context of the use of HPV testing as a primary screening test in the detection of cervical (pre-)cancer. We are also the first to report on HPV prevalence in a substantial sample of women with cervical cancer in East-Africa. We found HPV type distributions in cervical cancer that were similar to worldwide distribution, despite the fact that a different HPV distribution was found in the general population. In ICC, the same predominance was found for HPV16 and HPV18. This confirms the fact that these HPV types are very oncogenic and their prevalence increases as the severity of cervical lesions become worse [Franceschi and Clifford, 2005]. It also means that the prophylactic HPV vaccines would offer an overall similar, or only slightly lower protection than recorded elsewhere.

We also compared HPV type distribution in ICC in HIV-positive to HIV-negative women in by far the largest study sample reported to date. We found similar HPV type distributions, including HPV 16/18 prevalence in both groups. This is good news for the protection of future generations of HIV-positive women by HPV 16/18 vaccines.

There are high expectations for HPV vaccination to be more efficient in preventing cervical cancer in Africa, compared to the failing screening programmes. However, it should be kept in mind that any benefits from vaccination campaigns can only be expected 20 – 30 years later.

As a result of our findings, we formulated the following recommendations: 1) further research is needed on the importance of multiple-type infections in the carcinogenic process, especially for those containing HPV16 or -18, and the impact of vaccination against HPV16 or 18 in these instances; 2) careful monitoring of HPV-related cancers and intensified prevention programmes will be needed in those developing countries with high HIV prevalence and increased accessibility to ART; 3) the safety and efficacy of HPV vaccines in people with HIV still needs to be assessed; 4) the issue of appropriate cervical cancer screening and treatment algorithms for poor resource countries still needs further exploration, especially through demonstration projects in field conditions.
6. SAMENVATTING

6.1. Context en Objectieven

Achtergrond
In 2002 werden bijna 600.000 nieuw voorkomende kankers wereldwijd toegeschreven aan infectie met humaan papillomavirus (HPV) (d.i. 5.2% van alle kankers). Invasief cervixcarcinoom (ICC) was de meest voorkomende, waarvan 80% in ontwikkelingslanden met de hoogste incidentie in sub-Saharisch Afrika [Parkin et al., 2006]. De oorzaak van het merendeel van ICC wordt toegeschreven aan oncogene (hoog-risico) HPV types (HR HPV), waarvan de meest belangrijke types 16 en 18 geassocieerd zijn met ongeveer 70% van de ICC. Andere HR HPV types zijn minder oncogeen dan HPV 16 en 18. Naast de dominerende rol van HPV hebben andere factoren een invloed op het ontstaan van HPV-gerelateerde kanker, onder andere HIV-gerelateerde immunodepressie.

De belangrijkste recente ontwikkeling in het domein van HPV is de beschikbaarheid van profylactische vaccins regen infectie met HPV 16 en -18. Tegelijkertijd komt antiretrovirale behandeling in toenemende mate beschikbaar voor HIV-geïnfecteerde populaties in ontwikkelingslanden, in het bijzonder in sub-Saharisch Afrika. De toegenomen overleving van mensen met HIV zal echter ook de incidentie HPV-gerelateerde kankers doen toenemen.

Doelstellingen
De algemene doelstelling van dit werk is de beschrijving van de interactie tussen HPV en HIV, de epidemiologie van humaan papillomavirus bij Keniaanse populaties, de prevalentie van HPV types en de impact van HIV op invasieve cervicale kankers in Kenia.

Specifieke aandacht gaat naar 1) de beschrijving van de interactie tussen HIV, HPV en cervicale dysplasie en kanker in het HAART tijdperk; 2) de beschrijving van de prevalentie van HPV types in een centrum voor familiale planning in Nairobi; 3) de beschrijving van de leeftijdsspecifieke prevalentie van HPV types in een centrum voor familiale planning in Mombasa; 4) de beschrijving van de prevalentie van HPV types in invasieve cervicale kanker in Nairobi en de impact van HIV-besmetting op de distributie van HPV types bij deze vrouwen; en 5) de vergelijking van testkarakteristieken van verschillende alternatieve methodes van
screening voor het opsporen van cervicale precancereuze letsels en kanker in een onbemiddelde setting.

**Methode**

De data werden bekomen uit literatuuronderzoek en studies in Kenia (Nairobi en Mombasa) en Nicaragua. Deze studies werden uitgevoerd in samenwerking met de Universiteiten van Gent, Antwerpen en Nairobi, het Keniaanse Ministerie van Volksgezondheid, de Universiteit van Managua en het Ministerie van Volksgezondheid, Nicaragua en het Internationaal Agentschap voor Kankeronderzoek (WHO).

In Nairobi werd een studie uitgevoerd in een centrum voor gezinsplanning, waar een overzichtsonderzoek naar HPV gebeurde en waar de testkarakteristieken van alternatieve methodes voor screening werden vergeleken.

Teneinde de distributie van HPV types bij vrouwen met ICC te beschrijven, in functie van hun HIV status, werd een vergelijkend case-case onderzoek (vrouwen met ICC met en zonder HIV infectie) in het nationaal referentie ziekenhuis van Nairobi.

In het Rivas district in Nicaragua werden cytologie en VIA vergeleken in praktijkomstandigheden in een onderzoek naar opsporing van cervixkanker.

In Mombasa werd de leeftijd-specifieke prevalentie van HPV in de bevolking onderzocht via een leeftijd-gestratificeerde steekproef (100 personen per leeftijdsgroep van 5 jaar) uitgevoerd in een groep van vrouwen ouder dan 15 jaar die zich aanbieden voor gezinsplanning. Deze methodologie liet toe de resulaten te vergelijken met de IARC HPV populatie onderzoeken (IHPS).

In alle studies werd HPV getest op cervicale afstrijkjes via PCR technologie.

### 6.2. Resultaten

#### 6.2.1. HPV in Kenia

**In de algemene bevolking**

Een overzicht van HPV prevalentie werd uitgevoerd bij vrouwen die centra voor gezinsplanning bezochten, dit als benadering voor de algemene bevolking. De vastgestelde prevalenties van HPV behoorden tot de hoogste prevalenties die tot nog toe wereldwijd gevonden werden: 44.3% (30.8% HR) in Nairobi en 44.7% (34.6%) in Mombasa. In Nairobi werd, bij 369 vrouwen met een normale cervix, 38.8% (95% BI 33.8-43.9) HPV positief bevonden. De meest voorkomende
HPV types waren HPV52 (16.1% (10.5-23.1) van de positieve stalen), HPV16 en HPV66 (beide 9.1% (4.9-15.0)) en HPV31 (8.4% (4.4-14.2)). In Mombasa was, bij 560 vrouwen met een normale cervix, 43.2% (95% BI 39.1-47.4) HPV positief. De meest voorkomende HPV types waren HPV58 (22.3% (17.2-28.1) van de positieve stalen, HPV16 (15.3% (11.0-20.5), HPV52 (12.4% (8.5-17.2), HPV18 (9.9% (6.5-14.4) en HPV31 (9.1% (5.8-13.4). De prevalentie van HPV types in HPV positieve stalen van vrouwen met een normale cervix voor Nairobi en Mombasa werd vergeleken met de wereldwijde distributie, op basis van gegevens uit de IHPS analyse [Clifford et al., 2005a]. In tegenstelling tot de rest van de wereld, waar HPV16 tweemaal vaker voorkwam dan het tweede meest frequent HPV type (42), was dit niet het geval voor Nairobi of Mombasa, waar respectievelijk HPV52 en HPV58 ongeveer 50% vaker voorkwamen dan HPV16. Andere, wereldwijd meest voorkomende HPV types (18, 31), werden in gelijkaardige proporties gevonden in beide Keniaanse locaties. Deze resultaten tonen weer de variabiliteit van de prevalentie van HPV type aan, binnen verschillende populaties en zelfs binnen hetzelfde land.

Deze resultaten zijn belangrijk om de testkarakteristieken te beoordelen van methodes voor het opsporen van HR HPV bij vrouwen met een hoog risico van HSIL of kanker in deze regio. Voor Mombasa vonden we een zeer hoge prevalentie van HR HPV bij normale vrouwen (33.4%), met een beperkt toegenomen prevalentie bij vrouwen met een abnormale cervix (46.6%). Dit zou betekenen dat testen op HR HPV slechts een beperkt vermogen zou hebben om normale vrouwen te onderscheiden van vrouwen met abnormaliteiten, met een lage positieve predictieve waarde (12.6% voor Mombasa).

*Bij invasief cervixcarcinoom*

Onze studie draagt bij tot de groeiende kennis over de verdeling van HPV types bij ICC in Afrika. In Nairobi vonden we bij 153 HIV-negatieve vrouwen met ICC, een hoge prevalentie van infecties van het multiple type (13.7% (95% BI 8.7-20.2)). De meest voorkomende types HPV waren HPV16 (43.8% (35.8-52.0)), HPV18 (17.6 (12.0-24.6), HPV45 (17.0 (11.4-23.9) en HPV35 en HPV52 (beide 5.2% (2.3-10.0)). Vergeleken met de wereldwijde (WW) verdeling van de hierboven vermelde types bij ICC (Smith et al, 2007), toonden wij een lagere prevalentie van HPV16 aan (55.2% (54.2-56.2) WW), een hogere prevalentie van HPV35 (1.5% (1.2-1.8) WW), HPV45 (4.6 (4.1-5.2) en HPV52 (2.9 (2.5-3.3) WW). Eerder werden hogere prevalenties van HPV35, HPV45 en HPV58 vastgesteld in sub-Saharisch Afrika, vergeleken met Noord-Amerika en Europa [Clifford 2005a].
We vonden een graduele “verrijking” van HPV16 in een vergelijking van HPV verdelingen in normale tot pre-cancereuze en verder bij (HIV-negatieve) cancereuze letsels bij vrouwen uit onze 2 studies in Nairobi. HPV18 en 45 kwamen vooral voor in ICC (beide ongeveer 18% van positieve stalen). De veel voorkomende HPV types 35 en 52 in normale en pre-cancereuze cervices daarentegen, waren veel minder frequvent in ICC.

De impact van HIV

Een review van patienten dossiers van vrouwen met ICC, opgenomen in het nationaal referentieziekenhuis te Nairobi tussen 1989 en 1998 werd uitgevoerd. Er werd geen stijging waargenomen in incidentie van ICC over die periode, terwijl de achtergrond HIV prevalentie steeg van 5% tot 15%. Daarentegen vonden we in een case-control studie bij vrouwen jonger dan 35 jaar een 2.6 maal hogere kans op HIV infectie bij vrouwen met ICC en HIV, vergeleken met vrouwen met fibroieden in hetzelfde ziekenhuis. HIV-positieve vrouwen met ICC waren ook gemiddeld 10 jaar jonger dan HIV zonder ICC.

Het was niet verwonderlijk vast te stellen dat bij de HIV-positieve vrouwen uit de Nairobi FP populatie, 87% HPV-positief bleek te zijn, vergeleken met 39% bij de HIV-negatieve vrouwen. Omgekeerd bleek dat bij vrouwen met HR HPV, slechts 6% van de HIV-positieve een HSIL letsel had, vergeleken met 27% bij de HIV-negatieve vrouwen. Dit suggereert een zwakkere associatie tussen HR HPV en SIL bij HIV-positieve vrouwen, vergeleken met HIV-negatieve vrouwen. Dezelfde bevinding werd eerder gerapporteerd in Nairobi, zowel bij prostituees [Kreis et al., 1992] als bij een vergelijkbare populatie in een centrum voor familiale planning [Temmerman et al., 1999].

Wanneer de verdeling van HPV type bij ICC vergeleken wordt tussen HIV-positieve en HIV-negatieve vrouwen, was de meest opvallende vaststelling het toegenomen risico van multiple HPV infecties bij HIV-positieve vrouwen (respectievelijk 37.2% vs. 13.7%). Gelijkaardige bevindingen werden eerder gerapporteerd bij vrouwen met normale of precancereuze cervix voor HIV-positieve, vergeleken met HIV-negatieve vrouwen [Clifford et al., 2006b]. Een ondervertegenwoordigd zijn van HPV16, zoals vastgesteld bij de laatstgenoemde groep, werd in onze studie niet vastgesteld bij vrouwen met HIV met ICC. De enige types met een algemeen statistisch randsignificant verschillende distributie bij HIV-positieve vrouwen waren HPV45 (minder prevalent) en HPV52 (meer prevalent). De laatstgenoemde associatie werd eerder gemeld bij WHIV met hooggradige cervicale squameuze intra-epitheliale letsels (HSIL) (Clifford 2006). Wij vonden een gelijkaardig bereik van HPV types bij HIV-positieve,
vergeleken met HIV-negatieve vrouwen. Wat belangrijk is voor HPV vaccinatie: de prevalentie van HPV16/18 was vergelijkbaar in beide groepen. De helft van de WHIV in onze onderzoekspopulatie had een normale immuniteit (CD4+ > 500), wat een eerder recente HIV seroconversie suggereert. Een gevolg hiervan zou kunnen zijn dat het moeilijker wordt een verschil aan te tonen tussen HIV-positieve en HIV-negatieve vrouwen, daar het onwaarschijnlijk is dat HIV infectie het type HPV infectie beïnvloedt eenmaal het door HPV geïnduceerde cancereus proces irreversibel geworden is.

**6.2.2. Preventie van cervixkanker in Kenia door middel van HPV vaccinatie:**
Hoewel we een kleiner aandeel van HSIL geassocieerd aan HPV16 of -18 (38%) vaststelden, vergeleken met de wereldwijde distributie (52%) (Smith 2007), wat een lagere bescherming tegen HSIL in de Oost-Afrikaanse bevolking zou suggereren, toonden onze gegevens bij vrouwen met ICC een gelijkaardige prevalentie van HPV16 en/of -18 in HIV-positieve (64.7%) en HIV-negatieve (60.1%) vrouwen, en in het laagste segment van de wereldwijd gevonden deling [Smith et al., 2007]. Dit is goed nieuws voor zowel de Afrikaanse bevolking in het algemeen, als voor de vrouwen met HIV, hoewel het doel is om meisjes te vaccineren voordat ze seksueel actief worden en dus negatief voor HIV en HPV. De hoge kostprijs van het vaccin, evenals de vaccinatie van jonge adolescenten, zullen een specifieke uitdaging vormen in ontwikkelingslanden.

**6.2.3. Preventie van cervixkanker in Kenia door middel van alternatieve manieren van screening**
De performantie van visuele inspectie met azijnzuur (VIA) werd eerst onderzocht in een cross-sectioneel onderzoek bij 653 in een centrum voor familiale planning in Nairobi, Kenia. Om de drempel van HSIL of verder gevorderde ziekte vast te stellen, toonden de Pap uitstrijkjes een sensitiviteit en specificiteit van respectievelijk 83.3% (95% BI 71.5-91.7) en 94.6% (95% BI 92.3-96.4). Voor VIA bedroegen deze getallen 73.3% (95% BI 84.6-98.8) en 80.0% (95% BI 76.3-83.3), voor hoog-risico HPV 94.4% (95% BI 84.6-98.8) en 73.9% (95% BI 69.4-78.1) en voor cervicografie 74.5% (95% BI 59.7-86.1) en 89.9% (95% BI 86.5-92.6). De Pap test had een uitstekende performantie in de setting van het onderzoek, met een hoogopgeleide verpleegkundige en uitstekende cytologie afdeling voor het aflezen van de test. De goedkope VIA methode, die weinig technisch vereist stelt, presteerde ook adekwat.
De performantie van de VIA op het terrein, als screeningsmethode voor cervicale (pre)cancereuze letsels, werd onderzocht in Rivas, Nicaragua, bij 1,076 vrouwen, die tegelijkertijd onderzocht werden met VIA en Pap uitstrijkje [Claeys et al., 2003a]. Vier percent waren positief op zowel VIA als Pap, 25% op VIA alleen en 3% op het Pap uitstrijkje alleen. Tweemaal zoveel CIN2-3 en ICC werden gevonden door middel van VIA vergeleken met het Pap uitstrijkje. Hoewel, voor ieder extra gevonden casus, werden 8 ‘vals positieve’ gevallen gevonden, die ook moesten doorverwezen worden voor bevestiging. Dit is een belangrijk aspect, dat moet in overweging genomen worden in een omgeving met weinig middelen, waar er reeds een overbelasting is op vlak van doorverwijzing.

6.3. Besluiten en aanbevelingen

Onze onderzoeken zijn de eerste die de prevalentie van HPV types in een Oost-Afrikaanse populatie rapporteren. De prevalentie van HPV bevindt zich tussen de hoogste, wereldwijd gerapporteerd, met een ondervertegenwoordiging van HPV16 en een oververtegenwoordiging van HPV52 en -58. Deze informatie is belangrijk in de context van het gebruik van HPV testen als een primaire screeningsmethode in het opsporen van cervicale (pre)cancereuze letsels. Wij waren ook de eersten die de HPV prevalentie rapporteerden in een grote groep vrouwen met cervixkanker in Oost-Afrika. We vonden een verdeling van HPV types bij cervixkanker die vergelijkbaar was met de wereldwijde distributie, ondanks het feit dat een verschillende HPV distributie gevonden werd in de algemene bevolking. Bij ICC, werd hetzelfde overwicht gevonden voor HPV 16 en -18. Dit bevestigt het feit dat deze HPV types sterk oncogeen zijn en dat hun prevalentie toeneemt naarmate de ernst van de letsels groter wordt [Franceschi and Clifford, 2005]. Dit betekent ook dat de profylactische HPV vaccins een grotendeels gelijkwaardige, zij het lichtjes lagere bescherming zouden bieden als op andere plaatsen. We vergeleken ook de verdeling van HPV type bij ICC tussen HIV-positieve en HIV-negatieve vrouwen, in wat wereldwijd de grootste onderzoekspopulatie is tot nog toe. We vonden gelijkwaardige verdelingen van de HPV types, HPV 16/18 prevalentie in beide groepen inbegrepen. Dit betekent goed nieuws wat betreft de bescherming van de toekomstige generaties van HIV-positieve vrouwen door middel van HPV 16/18 vaccins. Er zijn hoge verwachtingen dat HPV vaccinatie effectiever zou zijn dan de falende programma’s voor screening, in de preventie van cervixkanker in Afrika. Echter, er mag niet
vergeten worden dat elke winst van een vaccinatiecampagne slechts 20-30 jaar later mag verwacht worden.

Als besluit van onze bevindingen, willen wij de volgende aanbevelingen doen: 1) verder onderzoek is nodig naar het belang van infecties van het multipele type in het carcinogene proces, specifiek voor infecties die ook HPV16 of -18 bevatten, en naar de mogelijk impact van vaccinatie tegen HPV16 of -18 in deze gevallen; 2) zorgvuldige opvolging van HPV gerelateerde kankers en geïntensifieerde preventieprogramma’s zullen nodig zijn in ontwikkelingslanden met een hoge prevalentie van HIV en een toegenomen beschikbaarheid van ART; 3) de veiligheid en werkzaamheid van HPV vaccins bij mensen met HIV moet nog aangetoond worden; 4) het probleem van een geschikte methode voor screenen naar cervixkanker en behandelingsalgoritmes voor ontwikkelingslanden moeten verder onderzocht worden, specifiek door onderzoeken op het terrein.
7. RÉSUMÉ

7.1. Contexte et Objectifs

Contexte
En 2002, près de 600,000 nouveaux cancers étaient attribués à une infection par papillomavirus humain (HPV) à travers le monde (i.e. 5.2% de l’ensemble des cancers) (Parkin et al., 2006). Le carcinome du col de l’utérus invasif (CCI) était le plus fréquent de ces cancers ; 80% de ces carcinomes se produisaient dans les pays en voie de développement où les incidences les plus fortes étaient trouvées en Afrique sub-saharienne. La majorité des ICC était considérée comme causée par des papillomavirus oncogéniques (dit à haut risque) (HR HPV), parmi lesquels les types 16 et 18, associés à près de 70% des cancers du col de l’utérus. D’autres types de HR HPV ont un potentiel carcinogénique mais dans une moindre mesure comparée aux types 16 et 18. Malgré le rôle prédominant des infections à HPV, d’autres facteurs jouent un rôle dans l’initiation des cancers liés à HPV ; un de ces facteurs est l’immunosuppression liée au Virus de l’Immunodéficience Humaine (VIH).

Un des domaines de développement les plus récents concernant HPV est la commercialisation de vaccins prophylactiques contre HPV-16 et -18. Par ailleurs, le traitement Anti-Rétroviral (TAR) est devenu accessible pour une plus grande proportion de populations infectées par le VIH dans les pays en voie de développement, plus particulièrement en Afrique sub-Saharienne. Cependant, l’amélioration de la survie de personnes séropositives au VIH va également augmenter le poids des cancers liés à HPV.

Objectifs
L’objectif général de ce travail est la description de i) l’interaction entre HPV et VIH, ii) l’épidémiologie du papillomavirus humain dans les populations Kényanes, iii) la prévalence des types de HPV, iv) l’impact du VIH sur les cancers du col invasifs au Kenya.

Nous nous sommes spécifiquement attachés 1) à décrire l’interaction VIH, HPV et dysplasie du col et cancer à l’ère de TAR ; 2) décrire la prévalence des types HPV dans un centre de planification familiale à Nairobi ; 3) décrire la prévalence spécifique par âge des types HPV dans un centre de planification familiale à Mombasa ; 4) décrire la prévalence de types de HPV chez les femmes atteintes de cancer du col invasif à Nairobi ainsi que l’impact de l’infection à
VIH sur la distribution des types HPV chez ces femmes ; 5) comparer les caractéristiques des tests de plusieurs méthodes alternatives de dépistage de lésions précancéreuses et cancéreuses dans des zones de faibles ressources.

**Méthodes**
A Nairobi, l’étude a été menée au sein d’une population fréquentant un centre de planification familiale, dans lequel une étude du HPV a été faite et les caractéristiques de tests de méthodes alternatives de dépistage ont été comparées.
Afin de décrire la distribution des types de papillomavirus chez les femmes atteintes de CCI, en fonction de leur statut vis à vis du VIH, une étude cas-témoins comparative (femmes atteintes d’ICC avec et sans VIH) a été conduite à l’hôpital national référent de Nairobi.
Dans la région de Rivas, au Nicaragua, la cytologie et l’Inspection Visuelle à l’Acide acétique (VIA) étaient comparées en pratique dans une étude de dépistage de cancer du col.
A Mombasa, une étude a été mené pour établir la prévalence spécifique par âge des types de HPV dans une population de femmes fréquentant le centre de planification familiale âgées de 15 ans et plus en utilisant une sélection stratifiée par âge (100 sujets par groupe d’âge de 5 ans), afin de comparer ces données à celles issues d’études du papillomavirus en population menées par le CIRC (IHPS).
Dans toutes les études, les tests HPV ont été effectués sur des prélèvements cervicaux à l’aide de la technologie PCR.

**7.2. Résultats**

**7.2.1. HPV au Kenya**

*Dans la population générale*
Les études de prévalence du papillomavirus ont été menées chez une population de femmes consultant le centre de planification familiale considérée comme une approximation de la population générale. Les prévalences de HPV étaient parmi les plus élevées au monde jusqu’à
ce jour : 44.3% (30.8% HR HPV) à Nairobi and 44.7% (34.6% HR HPV) à Mombasa. A Nairobi, parmi les 369 femmes avec un col de l’utérus normal, 38.8% (95% CI 33.8 - 43.9) étaient positive à HPV. Les types les plus fréquents étaient HPV52 (16.1% (10.5 – 23.1) des échantillons positifs), HPV16 et HPV66 (les deux 9.1% (4.9 – 15.0)) et HPV 31 (8.4% (4.4 – 14.2)). A Mombasa, parmi les 560 femmes avec un col de l’utérus normal, 43.2% (95% CI 39.1 – 47.4) étaient positives pour HPV. Les types de papillomavirus les plus fréquents étaient HPV58 (22.3% (17.2 – 28.1) des échantillons positifs), HPV16 (15.3% (11.0 – 20.5), HPV52 (12.4% (8.5 – 17.2) HPV18 (9.9% (6.5 – 14.4)) et HPV 31 (9.1% (5.8 – 13.4).

La prévalence des types de papillomavirus parmi les échantillons positifs pour HPV provenant de femmes avec un col normal a été comparée pour Nairobi et Mombasa avec les distributions mondiales obtenues à partir des IHPS (Clifford et al., 2005). Contrairement à de part le monde où HPV16 est deux fois plus prévalent que le second type le plus fréquent (42), ce n’était pas le cas à Nairobi ou Mombasa, où les types HPV52 et HPV58 étaient respectivement de 50% plus prévalent que HPV16. Les autres types les plus fréquents dans le monde (18, 31) étaient retrouvés dans des proportions similaires pour les deux sites Kényans. Ces résultats montrent à nouveau la variabilité des prévalences des types HPV entre les populations et même au sein d’un même pays. A Mombasa, nous avons observé une prévalence très élevée de HR HPV chez les femmes normales (33,4 %) avec une augmentation limitée de la prévalence chez les femmes ayant une malformation du col de l’utérus (46,6 %). Cela signifie que les tests HR HPV ont une capacité limitée dans la discrimination des femmes “normales” et des femmes présentant des malformations avec une valeur de prévision positive faible (12,6 % pour Mombasa).

En population atteinte de carcinome du col utérin invasif

Notre étude contribue à l’amélioration des connaissances de la distribution des types HPV parmi les femmes avec un ICC en Afrique. A Nairobi, parmi les 153 femmes avec un ICC, négatives au VIH, nous avons trouvé une forte prévalence des infections multiples (13.7% (95% CI 8.7 – 20.2)). Les types HPV les plus communs étaient HPV16 (43.8% (35.8 – 52.0)), HPV18 (17.6 (12.0 – 24.6), HPV45 (17.0 (11.4 – 23.9) et HPV35 et HPV52 (chaque 5.2% (2.3 – 10.0)). Comparée à la distribution des types mentionnés ci-dessus parmi les ICC dans le monde (Smith et al, 2007), nous avons montré une plus faible prévalence du HPV16 (55.2% (54.2 – 56.2) dans le monde), une plus forte prévalence des HPV35 (1.5% (1.2 – 1.8) dans le monde), HPV45 (4.6 (4.1 – 5.2) et HPV52 (2.9 (2.5 – 3.3) dans le monde). Auparavant, les plus fortes prévalence pour HPV35, HPV45 et HPV58 avaient été noté en Afrique sub-saharienne, comparées à celles d’Amérique du Nord et en Europe [Clifford and Franceschi, 2005].
Si l’on compare la distribution des types de HPV entre les situations normales, les lésions précancéreuses et les lésions cancéreuses (VIH négatives) parmi les participantes des deux études menées à Nairobi, nous observons un “enrichissement” graduel du HPV16. Les types HPV18 et 45 étaient notamment prévalents dans l’ICC (pour les deux, environ 18 % d’échantillons positifs). Inversement, les types HPV35 et 52 extrêmement prévalents dans les cols de l’utérus normaux et ceux portant des lésions précancéreuses étaient beaucoup moins fréquents dans l’ICC (pour les deux, 5,2 %).

*Impact du VIH*

Un examen des registres d’hôpitaux concernant les patientes atteintes d’ICC admises à l’hôpital spécialisé national de Nairobi entre 1989 et 1998 n’a révélé aucune augmentation de l’incidence de l’ICC, tandis que la prévalence de la VIH liée à l’environnement a triplé pour passer de 5 % à 15 % dans la population. Dans une étude cas-témoins ultérieure effectuée dans le même hôpital chez des femmes atteintes d’ICC, et de contrôles de fibromyomes, nous avons démontré que seulement chez les femmes de moins de 35 ans, celles atteintes d’ICC étaient 2,6 fois plus susceptibles d’être VIH positives que parmi les femmes ayant des fibromyomes. Les femmes VIH positives atteintes d’ICC étaient également en moyenne plus jeunes de 10 ans que les femmes atteintes d’ICC sans VIH.

Il n’était pas surprenant de retrouver, parmi les femmes positives pour le VIH dans la population des femmes du centre de planification familiale, 87% de femmes positives pour HPV, à comparer avec les 39% trouvés parmi les femmes négatives au VIH. A l’inverse, parmi les femmes à HR HPV, seulement 6% des femmes VIH-positives avaient une lésion précancéreuse de type HSIL, comparativement aux 27% parmi les femmes VIH-négatives. Ces résultats suggèrent une plus faible association entre HR HPV et lésions inflammatoires squameuses chez les femmes VIH-positives par rapport aux femmes VIH-négatives. Des résultats semblables ont été rapportées précédemment à Nairobi chez des femmes faisant commerce de leur corps [Kreiss et al., 1992], de même que dans une population comparable de centre de planification [Temmerman et al., 1999].

En comparant les distributions des types HPV chez les femmes ICC VIH-positives à celles chez les femmes VIH-négatives, le constat le plus marquant est le risque accru d’infections multiples à HPV chez les femmes VIH-positives (37.2%), comparé aux femmes VIH-négatives (13.7%). Des résultats semblables ont précédemment été rapportés chez des femmes avec des lésions précancéreuses ou cancéreuses pour les femmes VIH-positives, comparé aux femmes VIH-négatives [Clifford et al., 2006b]. La sous représentation de HPV16, comme signalé dans le
précédent groupe, n’a pas été retrouvée dans notre étude parmi les femmes avec VIH et ICC. Les seuls types avec une distribution différente à la limite de la significativité étaient HPV45 (moins prévalent) et HPV52 (plus prévalent). Cette dernière association a déjà été rapportée par le passé dans une étude portant sur des femmes avec des lésions squameuses de haut grade (HSIL) positives au VIH [Clifford et al., 2006b]. Nous avons trouvé une fourchette de valeurs semblables chez les femmes VIH-positives et VIH-négatives. Intéressant pour la vaccination contre HPV, la prévalence de HPV16/18 était similaire dans les deux groupes. La moitié des femmes positives dans le groupe étudié avait une immunité normale (CD4+ > 500), ce qui suggère une séroconversion récente. Ceci pourrait biaiser les résultats de la comparaison des femmes VIH-négatives et VIH-positives dans le sens d’une absence de différence, puisque de l’infection à VIH ne modifie vraisemblablement pas le type HPV à un stade irréversible du processus de cancérisation induit par HPV.

7.2.2. Prévention du cancer du col de l’utérus au Kenya grâce à la vaccination:

Bien que nous ayons trouvé une proportion plus faible de HSIL associées à HPV16 ou HPV18 (38%), en comparaison de la distribution dans le monde (52%) [Smith et al., 2007] qui suggérait une plus faible protection contre les lésions de types HSIL dans les population d’Afrique de l’Est, nos données parmi les femmes avec un ICC montrent une prévalence similaire de HPV16 et/ou HPV18 chez les femmes VIH-positives (64.7%) et VIH-négatives (60.1%), et à l’extrémité inférieure de la fourchette des valeurs répertoriées dans le monde. Ces résultats sont de bonne augure pour les populations africaines en général, de même que pour les femmes infectées par le VIH, bien que le but soit de vacciner les filles avant leurs premiers rapports sexuels, et donc alors qu’elles sont encore VIH et HPV négatives. Le coût élevé du vaccin, de même que la vaccination de jeunes adolescents posera des problèmes spécifiques dans les pays en voie de développement.

7.2.3. Prévention du cancer du col au Kenya grâce à des méthodes de dépistage alternatives

Les performances de la VIA a d’abord été étudiée dans une étude longitudinale suivant 653 femmes fréquentant le centre de planification de Nairobi, Kenya. Pour détecter le seuil de HSIL ou plus, des frottis cervicaux ont montré une sensibilité et une spécificité de 83.3% (95% CI 71.5- 91.7) et 94.6% (95% CI 92.3-96.4) respectivement. Pour la VIA, ces caractéristiques
étaient respectivement de 73.3% (95% CI 84.6-98.8) et 80.0% (95% CI 76.3-83.3), pour les HR HPV 94.4% (95% CI 84.6-98.8) and 73.9% (95% CI 69.4-78.1), et pour la cervicographie 74.5% (95% CI 59.7-86.1) and 89.9% (95% CI 86.5-92.6). Le frottis vaginal avait d’excellentes performances dans le cadre de la recherche, avec une infirmière qualifiée pour l’examen et une unité de cytologie performante pour la lecture de ce test. Le faible coût et le faible niveau technique de la méthode VIA était en adéquation avec l’environnement.
Les performances de la VIA sur le terrain comme test de dépistage de lésions cancéreuses et précancéreuses ont été déterminées à Rivas, Nicaragua chez 1,076 femmes dépistées simultanément par frottis vaginal et VIA. Quatre pourcent étaient positives pour VIA et frottis, 25% seulement en VIA et 3% seulement pour le frottis. Deux fois plus de carcinomes in situ stade 2 ou 3 (CIN2-3) et d’ICC ont été détecté grâce à la VIA en comparaison du frottis. Cependant, pour chaque cas supplémentaire détecté, 8 faux positifs étaient identifiés, justifiant la confirmation par un référent. Cet aspect est important à considérer dans les zones à faibles ressources généralement déjà surchargé au niveau des référents.

7.3. Conclusions et recommendations
Nos études sont les premières à rapporter la prévalence des types HPV dans les populations d’Afrique de l’Est. La prévalence de HPV est parmi les plus fortes dans le monde, avec une sous représentation de HPV16 et une sur représentation de HPV52 et 58. Cette information est important dans le contexte de l’utilisation de test pour HPV comme test de dépistage de premier recours des lésions cancéreuses et précancéreuses.
Nous sommes également les premiers à montrer la prévalence de HPV dans un échantillon substantiel de femmes avec un cancer du col de l’utérus en Afrique de l’Est. Nous avons trouvé des distributions de types HPV dans les cancers du col similaires à ceux rapportés au niveau mondial, malgré les différences de distribution de HPV dans les populations générales. La même prédominance pour HPV16 et 18 a été trouvée. Ceci confirme que ces types HPV sont particulièrement oncogéniques et que leur prevalence augmente avec la sévérité des lésions [Franceschi and Clifford, 2005]. Ceci signifie également que les vaccins prophylactiques contre HPV pourraient offrir une protection globalement semblable, à peine légèrement plus basse, que partout ailleurs.
Nous avons également comparé mes distributions des types HPV parmi les femmes ICC VIH-négatives et VIH-positives dans une étude de loin la plus large parmi l’ensemble des études rapportées jusqu’à présent. Nous avons trouvé des distributions de HPV semblables dans les
deux groupes, y compris pour les HPV16/18. Ceci constitue une bonne nouvelle pour la protection des générations à venir de femmes VIH-positives par les vaccins contre HPV16/18. De grands espoirs étaient placés dans la vaccination contre HPV pour être plus efficace pour la prévention des cancers du col en Afrique, en comparaison des échecs des programmes de dépistage. Néanmoins, il ne faut pas oublier que tout bénéfice de la campagne de vaccination ne pourra attendu que 20 à 30 ans plus tard.

En résumé de nos résultats, nous formulons les recommandations suivantes: 1) les recherches doivent être poursuivies sur l’importance des infections multiples dans le processus carcinogénique, spécifiquement les infections incluant HPV16 ou HPV18 ; et sur l’impact de la vaccination contre HPV16 ou HPV18 dans ce contexte ; 2) un recueil soigneux des cas de cancers liés à HPV, et des programmes de prévention intensifiés seront nécessaires dans les pays en voie de développement à forte prévalence du VIH et accès facilité aux traitements antirétroviraux ; 3) l’efficacité et la sûreté des vaccins HPV pour les personnes positives au VIH reste à démontrer ; 4) la question d’un dépistage du cancer du col approprié et d’algorithmes de traitement pour les pays à faibles ressources requièrent encore des explorations, particulièrement dans le cadre d’études sur le terrain.
8. ACKNOWLEDGEMENTS

This work would not have been possible without the contribution of the women who participated in these studies, health and support staff from the various clinics involved, the great study teams and collaborators in the field both in Nairobi and Mombasa, the continous support from the ICRH teams in Ghent and Mombasa. I am also most grateful for the contributions given by the Infections and Cancer Epidemiology group at IARC-WHO in the interpretation of the many data generated.

I am sincerely greateful to all.
9. REFERENCE LIST


202


203


Garcia F, Petry KU, Muderspach L, Gold MA, Braly P, Crum CP, Magill M, Silverman M, Urban RG, Hedley ML, Beach KJ (2004) ZYC101a for treatment of high-grade...
cervical intraepithelial neoplasia: a randomized controlled trial. *Obstet Gynecol* 103: 317-326


Hakama M, Miller AB, Day Ne, eds. Screening for cancer of the uterine cervix. Lyon, France: IARC Press; 1986. IARC Scientific Publication No. 76


expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. *J Immunol* 178: 3186-3197


206
conducted in a region of Costa Rica with a high incidence of cervical carcinoma.  
*Cancer* 87: 48-55  


207


cervical lesions surgically resected from HIV-infected women during follow-up of HPV infection. *Clin Infect Dis* 40: 451-457


Obwegeser JH, Brack S (2001) Does liquid-based technology really improve detection of cervical neoplasia? A prospective, randomized trial comparing the ThinPrep Pap Test with the conventional Pap Test, including follow-up of HSIL cases. *Acta Cytol* 45: 709-14


Palefsky JM, Berry JM, Jay N, Krogstad M, Da Costa M, Darragh TM, Lee JY (2006a) A trial of SGN-00101 (HspE7) to treat high-grade anal intraepithelial neoplasia in HIV-positive individuals. *Aids* 20: 1151-1155


Parkin DM, Ferlay J. et al., Cancer in Africa: Epidemiology and Prevention, IARC scientific Publications No. 153, 2003; p.133


Papanicolaou smears for cervical cancer screening in Morelos State, Mexico. *Cancer Causes Control* 14: 505-512


213


UNAIDS. Scaling up priority HIV/AIDS interventions April 2007 progress report


10. ANNEXES

10.1. ANNEX Curriculum Vitae

HUGO DE VUYST
17 Rue de la Ruche
69003 Lyon
France
Tel: +33 633528458
E-mail: hdvuyyst@yahoo.co.uk
Nationality: Belgian

PROFESSIONAL TRAINING
1992 Graduated as Medical Doctor from the Ghent University, Belgium.
1992 Medical training at the "Centre Hospitalier de Kigali" in Kigali, Rwanda, (internal medicine, gynaecology-obstetrics, paediatrics).
1992 Training course "HIV & AIDS", the clinical approach, at the Institute of Tropical Medicine, Antwerp.
1994-1996 Complementary training in General Practice.

WORK / EXPERIENCE
26 Nov 2006 – to date
Scientist at the International Agency for Research on Cancer (IARC – WHO). Development studies and analysis of the interaction HPV-HIV; collaboration HPV population surveys, scientific and technical writing and meta-analysis on non-cervical genital HPV-related cancers.
Consultancy International Agency for Research on Cancer (IARC – WHO). Development and site assessment multicentre study on HPV-type distribution and impact of HIV in sub-Saharan Africa.

Feb 2005 – May 2006
Epidemiologist Cervarix business unit GlaxoSmithKline Biologicals Rixensart, Belgium. Epidemiology HPV 16/18 vaccine: coordination regional epidemiology studies; epidemiology support to GSK departments: clinical trials (e.g. in HIV), commercial and R & D; leading studies in country-specific adolescent sexual behavior (age of sexual debut) and impact of HPV related cervical disease states on health related quality of life.

Jan 2004 – Dec 2005
Project Co-ordinator research project “Management of low grade cervical dysplasia in women with HIV”, a project funded by the FWO (Fund for scientific research Flanders) in collaboration with the Ghent University and the Antwerp University, project site Mombasa.

Oct & Nov 2003
WHO facilitator
- Workshop “Assessing Inflammation and Epithelial Integrity in Vaginal Product Research”, leading the sessions on methodologies of visual inspection of the vagina and cervix, Punta Cana, Dominican Republic, November 19\textsuperscript{th} - 21\textsuperscript{st}, 2003.
- Workshop “Cervical cancer screening by VIA: Principal Investigator’s workshop” at the Dept of Obstetrics and Gynecology, College of Health Sciences, University of Zimbabwe, Harare-Zimbabwe, 20\textsuperscript{th} – 24\textsuperscript{th} October 2003.

Nov 2001 – Dec 2005
Project Co-ordinator "Mombasa cervical cancer screening project", an EC funded INCO-DEV collaborative project between the Ghent University, Ministry of Health Kenya and several international institutions.

Oct 1997 – 2002
Project Advisor of the VLIR (Flemish Inter-university Council) Project "Training Clinical Cytology", a collaborative project between the Universitaire Instellingen Antwerpen/Belgium and the University of Nairobi.

Oct 1997 - Dec 2000
Project Field Co-ordinator, Principal Investigator of the VLIR "Project Reproductive Health", a collaborative research project between the Univ. of Ghent/Belgium and the Univ. of Nairobi/Kenya, focussing on cervical cancer, HPV prevalence and assessment of different screening options for cervical cancer in developing countries.

June 1997 - Sept 1997
Evaluation mission to the VLIR Project "Training Clinical Cytology" (see above), Nairobi/Kenya.

December 1996 - May 1997
Researcher at the International Centre of Reproductive Health (ICRH), Ghent.

December 1993 - November 1996
Clinician-researcher at the "Aids Clinical Research Unit" at the Institute of Tropical Medicine (ITM), Antwerpen.
Activities:
- outpatient care for people with HIV/AIDS and STD. Medical consultations.
- inpatient care on the AIDS/Tropical Medicine Ward of the University Hospital of Antwerp.
- epidemiological work: data analysis several surveys.
- co-ordination of projects: "counselling and partner information project".
- co-investigator in several Clinical Trials on HIV Treatments.
- travel medicine: medical consultations for travellers.
- training in HIV for Health Care Workers: teacher in a training course "HIV & AIDS, the clinical approach", ITM.

August 1994 - July 1995
Part Time general practitioner in private practice.

January 1994 - July 1995
Part Time practitioner in the “Centrum voor Seksuele Voorlichting, Gent” (Centre for Sexual Counselling), family planning and STD consultations.

October 1992 - November 1993
Clinical assistant at the "Aids Reference Centre" at the University Hospital of Ghent. Clinical follow-up of people with HIV, principal investigator on the A-APA Clinical Trial.

LANGUAGES
Mother tongue Dutch.
English and French fluently spoken and written.
Elementary knowledge of Spanish, German and Kiswahili.

SCIENTIFIC ACTIVITIES


2003 Facilitator as WHO temporary advisor at the workshop on "Assessing Inflammation and Epithelial Integrity in the Vaginal Product research", Punta Cana, Dominican Republic, November 19-21, 2003.


Member of the organising committee of the “East and Southern Africa Regional Meeting on the Prevention and Control of Cervical Cancer” in Nairobi, March 1998.


5th European Conference on Clinical Aspects and Treatment of HIV Infection, Copenhagen - Denmark, 26.09.’95 - 29.09.’95 Poster presentation.


9th International Conference on AIDS / 4th STD World Congress, Berlin, Germany, 6-11.06.1993.


PUBLICATIONS


Oral Presentations


223


Claudia Nascimento, Gary Clifford, Hugo DeVuyst, Silvia Franceschi.  HPV prevalence and type distribution in carcinomas and intraepithelial neoplasia lesions of the vulva and vagina. The first in a series of meta-analysis in anogenital sites other than cervix. 24th International Papillomavirus Conference, Beijing, 3-9 November 2007.

**Poster Presentations**


R. Colebunders, Y. Fleerackers, H. De Vuyst, A. Deroo, P. Simons. What is safe sex? Opinions of homosexual and heterosexual persons with HIV infection in Flanders. Seventh European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 26-30.03.95.


10.2. ANNEX Supporting Paper

Integration of cervical screening in family planning clinics.

*Claeys P, De Vuyst H, Mzenge G, Sande J, Dhondt V, Temmerman M.*

Special communication

Integration of cervical screening in family planning clinics

P. Claeyss, H. De Vuyst, G. Mzenge, J. Sande, V. Dhondt, M. Temmerman

*International Centre for Reproductive Health (ICRH), Department of Gynecology and Obstetrics, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

†Family Planning Association of Kenya, Nairobi, Kenya

‡Department of Medical Microbiology, Faculty of Medicine, University of Nairobi, Nairobi, Kenya

§Second Cycle Medicine, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Received 22 October 2002; received in revised form 13 January 2003; accepted 15 January 2003

Abstract

Objectives: To assess the suitability of cervical cancer screening in family planning (FP) clinics and the relevance for women’s health. Methods: A survey was done on clients visiting the clinics of the Family Planning Association of Kenya (FPAK). Client characteristics, age, screening status and PAP smear results were registered. In-depth interviews were held with a limited number of staff and clients. Results: In 1999, 38 052 clients visited FPAK clinics, 43.5% were younger than 30 years old. More than 10 000 cervical smears were taken. A total of 4.5% of the smears were abnormal, including 1.5% high-grade squamous intraepithelial lesions (HSIL) and 0.2% invasive cancers. The clinics were well prepared to provide high quality screening services. Patients and staff had a positive view on screening. Conclusions: Providing cervical cancer screening in FP clinics is beneficial for the clients but is unlikely to have an impact on the epidemiology of cervical cancer morbidity as FP services reach only a small percentage of the women who are most at risk. Measures to reach more and older women could assure a larger impact.

Keywords: Cervical cancer; Family Planning clinics; Integration

1. Introduction

Cervical cancer is responsible for more than 234 000 deaths per year. Its prevention remains an issue in low resource settings [1]. Screening programs resulted in a dramatic decrease of cervical cancer morbidity and mortality in Western countries [2]. Most developing countries have not introduced cervical screening programs, because of limited resources and competing health priorities. Where these programs do exist, problems have been reported with coverage, logistics, maintenance of equipment, training and follow-up [3].

In high-risk sub-Saharan African countries, screening for cervical cancer is not done in a routine way, although it can be provided on demand in certain settings [4]. In Kenya, where the incidence of cervical cancer is estimated at
45/100,000, only 6% of women presenting with invasive cancer at Kenyatta National Hospital have a history of previous screening [5]. In this country, cervical cancer screening is mainly offered through family planning (FP) clinics. With this assessment, we found out how suitable FP clinics are for providing screening and what the relevance is of this screening for women's health and cervical cancer morbidity.

2. Methodology

The assessment was done in clinics of the Family Planning Association of Kenya (FPAK). Data were collected from November 1999 until February 2000. Information on the clinics, the organization of the screening services, the perceived problems, the attitudes towards screening and the influence of the screening program on the workload of the staff was obtained through interviews with key persons at FPAK headquarters, including the Program Manager, the lab technologist in charge and the Senior Program Officer for service delivery. Data on client characteristics were obtained through a review of 791 files. These files were selected through systematic random sampling of women attending seven of the 14 FPAK clinics in 1999. In four of these clinics interviews were conducted. Two urban clinics (Ribeiro, Phoenix) and two rural clinics (Meru and Kisii) were selected. There, four female head nurses, eight other service providers (including two males) and 84 randomly chosen patients were interviewed through exit interviews. Results of Papanicolaou (PAP) smears taken in 1999 were retrieved from the central data handling system of FPAK. More detailed data on age, parity, and contraceptive use of all women with abnormal PAP smears were collected from records at the cytology department. This was also done for a random sample of 981 of the 10,335 women who had normal smear results during that same year. To assess the age distribution of women from whom a PAP smear was taken, the data from women with a normal smear were weighted with a factor of 10.535.

3. Results

3.1. Description of the family planning clinics and clients

FPAK is a local Non-Governmental Organization (NGO) with a countrywide network of 14 clinics, distributed over eight urban and six rural communities. FPAK clinics are typical examples of the evolution of family planning clinics from previously being distributors of FP methods to currently having developed into women's health clinics, providing a wide range of services, including cervical cancer screening. In 1999, 38,052 clients of whom 5,135 new clients, visited the FPAK clinics, on average three times a year. The mean age of the clients was 31.0 years (median 30) of age, with respectively 43.5% younger than 30 and 10.5% older than 39 years of age. The main reasons for attending the clinic were family planning services and cervical cancer screening (Table 1).

3.2. Organization of the screening services

The policy at FPAK is to perform a PAP smear on a yearly basis on all clients. Screening services are available in all the FPAK clinics. They are all fully equipped and dispose of the necessary supplies to do PAP smears. All smears are sent to the central laboratory in Nairobi, which is at a maximum distance of 500 km. There they are processed and read by two cytotechnologists and one cytopathologist. The transport of slides as well as results is done by courier services, which reach any place in the country within 1 day. All results are stored in a computerized information management system. On average, the clinics receive the results within 2–4 weeks.

All clinics have trained nurses, who take an average of 10 PAP smears a week (range 4–37). Promotion for cervical cancer screening is done through posters in the waiting room, through personal communication of providers to clients, and to some extent by the community-based distributors (CBD) of FP methods in rural and slum areas. PAP smears are usually not free of charge. Interviewed patients paid on average 200 KSH
(range 0–300), depending on the location and the socio-economic level of the patient. This user’s fee, which is considered high by 16.3% of the women, does not cover the total screening cost (e.g. the kits are donated).

When a dysplasia is diagnosed, patients are referred to medical doctors in public or private hospitals. No information is available on patient compliance or on the number of patients actually treated.

3.3. Prevalence and characteristics of women with dysplasia

In 1999, 10 830 PAP smears were done and processed, half of them in women of 25–34 years of age and nearly 36% in women younger than 30 years old. The mean age of the women was 32.9 years of age (median 32). The quality of smear taking was good: only 0.38% was reported as unsatisfactory. A total of 4.5% of smears were abnormal: 2.8% showed a low-grade squamous intraepithelial lesions (LSIL), 1.5% a high-grade squamous intraepithelial lesions (HSIL) and 0.2% invasive cancer. The prevalence rate of HSIL was 0.7% for women younger than 30 years of age, 1.7% for women between 30 and 35 years of age and more than 2% for women 36 years of age or older. The prevalence rate of cervical cancer was 10 times higher in women of 40 years of age or older, compared with the other age groups (Table 2).

Women with HSIL and invasive cancer were significantly older and had significantly more children than women with normal PAP results, but the age and parity of women with LSIL did not differ from women with normal PAP smears (Table 3).

3.4. Screening status and barriers to screening

All interviewed nurses declared to promote annual PAP smears to all their clients, independent of the age or screening history of the woman. Fifty percent of the interviewed patients declared not to have been screened in the last 3 years. Women of 30 years of age and older were screened significantly more than women younger than 30 years of age; 36/48 (75%) and 14/36 (38.9%), respectively ($P=0.01$).

Of the women ever screened, 96% of them had their PAP test done in a FPAK clinic. The main reasons mentioned for this choice were quality of service delivery and comprehensive approach. Service providers considered lack of awareness and knowledge, as the main barrier to screening.

| Table 1 Characteristics of clients attending the family planning clinics |
|------------------------|------------------|
| Age group (n=791)     |
| <25                   | 125 (15.8)       |
| 25–29                 | 219 (27.7)       |
| 30–34                 | 239 (30.2)       |
| 35–39                 | 125 (15.8)       |
| ≥40                   | 83 (10.5)        |
| Reasons for attending (n=788) |
| FP services          | 592 (75.2)       |
| PAP smear            | 124 (15.7)       |
| Curative services    | 7 (0.9)          |
| Counseling           | 42 (5.3)         |
| Antenatal clinic     | 4 (0.5)          |
| Infertility management | 3 (0.4)        |
| Other reasons        | 16 (2.0)         |
| Current use of family planning (n=789) |
| None                 | 126 (16.0)       |
| Norplant             | 75 (9.5)         |
| Oral contraceptives  | 178 (22.6)       |
| Depo-Provera         | 253 (32.1)       |
| Intrauterine device  | 97 (12.3)        |
| Tubal ligation       | 41 (5.1)         |
| Vasectomy            | 4 (0.5)          |
| Condoms              | 10 (1.3)         |
| Foaming tablets      | 5 (0.6)          |
| Professional status (n=82) |
| Employed             | 51 (62.2)        |
| Housewife            | 29 (35.4)        |
| Student              | 2 (2.4)          |
| Educational level (n=84) |
| Primary              | 21 (25.0)        |
| Secondary            | 33 (39.3)        |
| Higher level         | 30 (35.7)        |
| Personal monthly income (n=80) |
| None                 | 31 (38.7)        |
| 1–5000 KSH           | 16 (20.0)        |
| >5000 KSH            | 33 (41.3)        |
| Screening status (n=84) |
| Adequately screened  | 42 (50.0)        |
| Screened >3 years ago | 8 (9.5)         |
| Never screened       | 34 (40.5)        |

Data obtained through revision of files. Data obtained through interviews with clients.
This was confirmed by the clients’ interviews. Sixteen out of 34 (47%) women mentioned lack of awareness as the reason for not being screened, 10 (23.5%) implied negligence, 4 (11.8%) had economic reasons and the others said absence of medical problems and fear of the test result motivated their conduct.

Twenty four out of 47 (51.1%) women with an income of ≤5000 KSH had never been screened compared with eight of 33 (24.2%) of those with an income of 5000 KSH and more (OR = 3.26, 95% CI 1.22–8.69). After adjusting for age, educational status and employment, low income remained a risk factor for absence of screening (AOR 2.82; 95% CI 1.02–7.81).

4. Discussion

From a programmatic point of view, offering cervical cancer screening in MCH/FP services seems to be a waste of resources, as older women, most at risk for cervical cancer, are poorly covered by these services [6]. Some people argue that in developing countries, cervical cancer occurs at an earlier age, so screening should start earlier. Two studies on invasive cervical cancer in Kenya showed the mean age of the patients to be 42 and 47 years of age, respectively [7,5]. In our study population, the mean age of invasive disease is nearly 52 years of age, but HSIL present at a mean age of 36 years of age. The timely detection and treatment of this precursor stage should be the aim of all screening programs. The maximum age-related benefit is obtained by starting the screening at the age of 35 [8]. The mean age of the FPAK clients was 31 and more than half of the clients were 30 years of age or older, thus being a suitable population for cervical cancer screening. Under the age of 25, only 0.4% of FP attendees were diagnosed with HSIL, but the prevalence was four to five times higher for women aged 30 and more. This supports the thesis that screening young women should be discouraged [9]. Screening services can be made more efficient by starting screening at 30 years, and by increasing the screening...
interval to 3 years, as the additional benefit gained by screening more frequently is very small [9,10].

The integration of cervical cancer screening services within FP clinics is in line with the concept of reproductive health services as endorsed by the Cairo Conference of 1994. During the last decades, family planning clinics worldwide have broadened the scope of their services, evolving from ‘providers of contraceptives’ to ‘women’s health clinics’, providing a broad range of preventive and curative services. FP clinics have access to a population of sexually active women. They have the necessary infrastructure, trained personnel and the logistics to provide services of good quality. As gynecological examinations are routine clinical practice in FP clinics, the screening of women at risk for cervical cancer is an opportunity not to be missed. Our assessment of FP AK outlines additional advantages of these clinics such as their good reputation, their geographical accessibility, their equipped laboratories, and their highly motivated staff.

An additional element advocating for the screening of FP attendees is the suggested association between oral contraceptives and cervical cancer. A pooled analysis of eight case-control studies showed that the risk of cervical cancer increased three-fold in HPV positive women who had been using oral contraceptives for 5 years or longer [11]. Amongst the FP AK clients, 22.6% used oral contraceptives. In our assessment, we did not gather information about the duration of use, but the regular screening of all FP attendees aged 30 and older guarantees that the group of long-term users is surely not missed.

Linking cervical cancer screening to contraceptive use does, however, contain risks and limitations. The first is that potential FP users might be deterred from using contraceptives. This could particularly be the case if a pelvic examination is required prior to provision of hormonal contraception. The reinforcement of the perception that hormonal contraceptive methods are dangerous could further discourage women from using them. As a result, the access to highly effective contraceptive methods might be reduced and women’s overall health risks increased [12]. A group of women who might adopt this approach are women served by CBD, as they do not attend the clinics regularly. The CBD network can, however, also be used as a very efficient means of promoting cervical cancer awareness and screening in the communities. They are excellent mediators for health promotion and have access to those women who do not easily attend reproductive health services, especially older women and women from lower socio-economic classes.

Another risk is that cervical cancer is perceived as related to reproduction and therefore that screening activities stop after menopause sets in. Both service providers and clinic attendees would then focus on over-screening young and sexually active women, while the older, most at risk population is not reached. This does not seem the case in FP AK clinics, as older women were significantly more screened than younger ones. The age distribution of the clients does, however, indicate that women do not attend FP clinics regularly after menopause. One could overcome this problem by reinforcing the focus of the educational messages in the clinics on screening at older age. The organization of well women’s consultations, where women can attend for whatever reproductive health related issue, is an additional option.

A third limitation is related to the nature of family planning clinics in general. As NGO clinics, their services have to be sustainable, and user’s fees have proven an important barrier for the poorest population. Our data show that the women attending FP AK clinics are not representative for the overall population of Kenya. In this group of middle-class, high-educated women, economic problems were still mentioned as the main barrier to screening by one out of six women who had not been screened in the past. It is difficult to strike the balance between safeguarding quality and financial accessibility. Here again, outreach activities can be a means of reaching underserved populations, who could be exempted of users fees.

In conclusion, our assessment has shown that cervical cancer screening based on PAP smear tests can be perfectly integrated in FP clinics. This impacts positively on clients’ health as women with precancerous lesions are treated timely and others are reassured. It is unlikely, however, that there is an epidemiological impact on morbidity.
due to cervical cancer as FP services reach only a small percentage of the women most at risk. The impact could be improved by organizing information and education sessions on cervical cancer, by providing screening services as part of the community outreach activities and by taking initiatives to reduce financial barriers. A more rational organization of screening, concentrating on women of 30 years of age and older and on a tri-annual basis can further reduce the costs.

References

10.3. ANNEX Supporting Paper

HPV vaccines in HIV-positive men and women

De Vuyst H. and Franceschi S.


The references used in this paper are listed at the end of this section.
ABSTRACT

Purpose of the review

People living with HIV (PHIV) are at higher risk for human papillomavirus (HPV)-related cancers. The purpose of this report is to assess the potentials and limitations of vaccines against HPV in HIV-positive women and men.

Recent findings

A worldwide meta-analysis of published data established the under-representation of HPV16, and increased prevalence of multiple-type HPV infections in HIV-positive women. In addition, studies from sub-Saharan Africa showed associations between HIV-related immunodepression and progression of HPV infection to cervical lesions, and an increased risk for cervical cancer in women with HIV. More data were published on HPV infection in anal and vulvar/vaginal neoplasia and the increased incidence of these neoplasias in PHIV. A prophylactic vaccine against HPV6, 11, 16 and 18 has been licensed and one against HPV16 and 18 is under investigation. Both have shown high efficacy against persistent infection, as well as related cervical lesions with the included HPV types for up to five years. Promising results were also reported on therapeutic vaccines, notably for the treatment of cervical intraepithelial neoplasia grade 2 and 3. One study was in HIV-positive men with anal lesions.

Summary

Safety and efficacy of HPV vaccines in PHIV need to be assessed in order to prevent this infection in current and future generations. Meanwhile, screening of HPV-related cancer among PHIV should be put in place.

Keywords: Human papillomavirus, HIV, vaccines, anogenital cancers
Introduction

In 2002, nearly 600,000 new cancers were attributed to human papillomavirus (HPV) infection (i.e., 5.2% of all cancer) worldwide [1*]. Cervical cancer was the most common, and in the vast majority considered to be caused by HPV [2]. HPV infection is also associated with cancer of the vulva/vagina, anus, penis, and a small fraction of the cancers of head and neck [3]. HPV16 and 18 have been found to predominate in cervical cancer (>70%) [4**], and to an even greater extent in the other cancer sites [3]. Other HPV types, called high-risk types, have oncogenic potential but to a lesser degree than HPV16 and 18. Despite the predominant role of HPV, other cofactors also play a role in the onset of HPV-related cancers, one of which is HIV-related immunosuppression.

The most important recent development in the field of HPV is the availability of a prophylactic quadrivalent vaccine against infection with HPV6, 11, 16, and 18 [5**]. At the same time, on the side of HIV infection, anti-retroviral treatment (ART) is becoming accessible to increasing proportions of HIV-infected populations not only in developed, but also in developing countries, most notably sub-Saharan Africa (2006 Report on the global AIDS epidemic. Available at: http://data.unaids.org/pub/GlobalReport/2006/200605-FS_globalfactsfigures_en.pdf 2006). Hopefully, this will result in better control of opportunistic infections in all countries. Improved survival will, however, also increase the burden of HPV-related cancers.

Immunological aspects

HPV infects basal cells from mucosal epithelia and starts its life cycle with limited expression of “early” (E) viral genes, most notably E6 and E7, which are potent viral oncoproteins that immortalise human cell types through inactivation of tumour suppressor proteins [6*]. As daughter cells move to the suprabasal layers, the cell continues to replicate HPV genomes and starts to express “late” (L) viral genes. L1 and L2 are viral capsid proteins that self-assemble into icosahedral capsids containing the viral genome. L1 is the main capsid and also the largest and most immunogenic.

In natural infection, HPV uses several methods to keep itself “invisible” thereby avoiding host immune response [6*,7*]. There is no viremia, and dendritic cells are not triggered as the infection is non-lytic and the virus uses molecular mimicry (sequence similarity of parts of viral proteins and host proteins) [8]. In addition, HPV is able to down-regulate the
expression of host interferon genes, with decreased innate and adaptive host immune responses [7*,9*].

There is some evidence to suggest that the most common and carcinogenic HPV type, HPV16, has additional molecular mechanisms to evade clearance by the immune system [10,11,12]. Nonetheless, 70 to 80% of HPV infections are eventually cleared in one year’s time [10,12]. Natural immunity against HPV involves innate and adaptive immune responses, predominated by a strong localised cell-mediated immunity, which is associated with lesion regression and the generation of serum neutralising antibodies [7*]. The presence of lymphoproliferative cell-mediated immune responses to E7 correlated significantly with regression of cervical intraepithelial neoplasia (CIN), clearance of HPV infection [13], and better prognosis in early-stage cancers [14].

**Impact of HIV on HPV infection**

HPV infection is predominantly acquired through sexual intercourse, as is HIV, although in some parts of the world substantial fractions of people have acquired HIV through contaminated needles and blood. It is thus not surprising that a very large proportion of PHIV are also infected with HPV [15**]. HIV, however, also affects unfavourably the natural history of HPV infection. Clearance of HPV infection is diminished among immunocompromised individuals [11,16], and progression to neoplastic lesions becomes more probable.

Several studies have found a broader range of both high- and low-risk HPV types in HIV-positive compared to HIV-negative women [17,18,19]. In a recent meta-analysis on 20 studies including 5,578 women with HIV worldwide, HPV16 accounted for a smaller proportion of HPV infections in HIV-positive women than the general female population. This was also the case in women with high-grade squamous intraepithelial lesions (HSIL) [15**]. Conversely, other types (high-risk types 18, 51, 52, 58 and low-risk types 11, 53, 61) were more frequently detected in HIV-positive women with HSIL. The HERS study showed that HPV16 was more weakly associated with immune status than the other HPV types (i.e., control of HPV 16 is also a challenge for immunocompetent women) [11]. In addition, HIV-positive women with or without cervical lesions also harbour many more multiple-type HPV infections, compared to the general female population [15**]. Although it is unclear whether multiple-type infections pose a higher risk for CIN or invasive cancer than single-type infections, these facts further complicate the study of HPV in HIV-positive women.
HPV and HIV in cervical neoplasia and cancer

Squamous intraepithelial lesions (SIL) of any grade are consistently more prevalent in HIV-positive than HIV-negative women in Western [20,21] and African countries [22,23], with increased incidence and progression rate of SIL [24,25,26**].

The prevalence and incidence of SIL increases among HIV-positive women with a decline in CD4 counts [25,27,28]. Incidence rates in less-immunocompromised women (CD4 count >500/µl) appeared similar to HIV-negative women [29]. A recent study confirmed earlier findings that HPV infection takes longer to clear in HIV-positive women [30*].

Although early studies on the association between HIV and invasive cervical cancer showed somewhat inconsistent findings [2,31], it has become increasingly clear that HIV-positive women are at increased risk for invasive cervical cancer [32,33]. The relative risk of invasive cervical cancer among HIV-positive women, however, varies from one country to another, and depends largely on competing causes of premature death (e.g., in sub-Saharan Africa) [34,35**] or the prevention of progression of pre-invasive lesions due to early detection in screening programs (e.g., the United States) [33]. It seems, however, that HIV-induced immune impairment does not affect the progression from in situ carcinoma to invasive cervical cancer, as associations of the same magnitude were found for the two diseases in the United States [33].

For an excess of invasive cervical cancer and other HPV-related malignancies of the anogenital tract to occur, extreme degrees of immunosuppression are not required. Indeed, contrary to Kaposi’s sarcoma (KS) and non-Hodgkin lymphoma (NHL), the majority of cervical cancer have been reported in women with CD4 counts >200/µl [36]. This is probably the reason why the advent of highly active antiretroviral therapy (HAART), and consequent partial immune reconstitution, has had little or no impact on the incidence of invasive cervical carcinoma, while it has curbed KS and NHL incidence.

Anal HPV infection and anal neoplasia in PHIV

Anal HPV infection is very frequent in PHIV, and it was found in 78% of HIV-positive men in one cohort study [37]. Whereas receptive anal intercourse is a strong risk factor for anal HPV infection in HIV-negative men [38], anal HPV infection is also very frequent in HIV-positive men who do not report engaging in anal intercourse (46%) [39].

Similar to cervical cancer, anal cancer is associated with HPV infection [40], especially HPV16, which one study found in 73% of anal cancers [41]. Anal cancer was reported to be
more frequent in men having sex with men (MSM) before the HIV epidemic [42], but currently anal cancer has been found more frequently in both men and women with HIV, and incidence continued increasing in the post-HAART era [43,44**]. Consistent with findings on cervical cancer, anal carcinoma has been found to be associated with duration of HIV infection, but not with degree of immunosuppression [45].

**Vulvar/vaginal HPV infection and neoplasia in HIV-positive women**

The majority of vulvar and vaginal neoplasias are also associated with HPV infection. A recent study found HPV DNA in approximately 90% of biopsies of vulvar and vaginal intraepithelial neoplasia (VIN/VAIN) grades 2 and 3. Sixty percent of vulvar cancers were found to harbour HPV, with a higher HPV prevalence in younger women (<56 years). HPV16 and 18 were the predominant types [46**]. More recently, HPV 16 prevalences of 73% in VIN and 60% in VAIN were reported [47*].

Cohort studies have shown increased incidence and prevalence rates of precancerous lesions of the vulva and vagina in HIV-positive women [48,49*]. HIV-positive women are also at increased risk of developing *in situ* and invasive carcinoma of the vulva and vagina, compared to HIV-negative women [33].

**Prophylactic HPV Vaccines**

Currently, two prophylactic vaccines, based on HPV virus-like particles (VLP), which are generated by the synthesis *in vitro* of L1 proteins, have been developed and tested in extensive clinical trials. One quadrivalent vaccine against HPV6, 11, 16 and 18 (Gardasil, MSD) is commercially available in the United States and in many other Western countries. The other, a bivalent vaccine against HPV16 and 18 (Cervarix, GSK), is under evaluation. The duration of vaccine efficacy is unknown, but antibody levels have also been shown to stay high for at least 5 years [50**], as have preventive effects against persistent infection and cervical lesions caused by the HPV types included [51**]. Some cross-protective effect of the bivalent HPV16/18 vaccine against incident infection with HPV45 and 31 has also been reported [51**].

As these vaccines are prophylactic, it is assumed that maximum effectiveness will be achieved by administration before exposure to HPV. However, as long as duration of protection
is unknown, it seems unwise to vaccinate small children and therefore, the current recommendation is to vaccinate female adolescents aged 9-12 years [52**].

Studies among males 9-15 years have shown immune responses to the quadrivalent vaccine that are similar to those in females [53**], however no information is yet available on the efficacy of HPV vaccines against infection and HPV-related lesions in males. The anatomical characteristics of the male anogenital epithelia may make antibodies against HPV less effective than in the female genital mucosa.

**Therapeutic vaccines**

Therapeutic vaccines target already established HPV infections and the associated precancerous or cancerous lesions. This would benefit individuals of a much broader age range than prophylactic vaccine, and this is especially true for PHIV who often are concurrently infected by both viruses.

Most efforts have focused on eliciting cytotoxic T lymphocyte responses against E6 and E7. Therapeutic vaccines present far more challenges than prophylactic vaccines, and indeed there is nearly no precedent in the cancer field. In respect to HPV, only data from phase I/II clinical trials are available.

So far, the most significant results obtained were with a HPV16 E7 *Mycobacterium Bovis* heat shock 65 (HSPE7) fusion protein vaccine with 23% complete response in 58 women with CIN3 (http://www.nventacorp.com/news/pr20070305_Einstein_SGO.htm last accessed 26/03/07). An MVA E2 recombinant vaccinia virus used in 34 patients with CIN2/3 [54*] showed 59% total response. A randomised controlled trial with DNA vaccine encoding fragments from HPV16/18 E6 and E7 (ZYC101a) including 86 vaccinated and 41 control subjects with CIN2/3 [55] (http://www.mgipharma.com/wt/page/amolimogene), found a significantly higher resolution of lesions only in women younger than 25 years (70%) versus a control group (23%). One study used HSPE7 in different dosages in 15 HIV-positive men with high-grade anal intraepithelial (AIN) lesions, in which five lesions regressed. There were no adverse effects on changes in HIV viral load and CD4/CD8 ratio [56*].

**HPV vaccines in HIV-infected people:**

Prophylactic vaccines work mainly through humoral immunity, which is relatively well preserved in PHIV before severe immunosuppression. Even if there are reasons to expect
adequate response to HPV vaccination in PHIV [57], many would not benefit from a prophylactic vaccine as they would have already been infected with the HPV types present in the vaccine. Therefore, the highest benefit could be expected from vaccinating young adolescents in populations with high HIV prevalence, thus also protecting future generations of PHIV.

However, as HPV VLP vaccines are highly immunogenic with elevated antibody levels, but also stimulate innate (58) and cellular immunity, it might be possible that vaccinating PHIV could offer a boosting effect of previously acquired natural immunity [5,50**], an effect that has not yet been elucidated even among HIV-negative women. Studies on immunogenicity and clinical effectiveness in PHIV are still needed. Experience with other vaccines is rather encouraging, although increased dosages or altered administration schedules might be required in PHIV. One study with recombinant hepatitis B virus (HBV) vaccine in adult PHIV showed significantly better seroconversion rates for double dose (47% of 98 vaccinated), compared to standard dose (34% of 94). Rates were also significantly higher in participants with CD4 count ≥350/µl [59]. Addition of an immunostimulating adjuvant to HBV vaccine resulted in 100% seroconversion rate at 12 months in 19 vaccinated adult PHIV under ART treatment [60]. A study with tetanus toxoid booster in 15 HIV-positive children and young adults, all on HAART, demonstrated diminished though sufficiently preserved anamnestic responses. [61*] The authors suggested that shorter booster intervals might be considered in PHIV.

The safety of VLP vaccines in PHIV is currently being investigated. Concerns are not very severe, however, as these are non-live vaccines and other such vaccines (e.g. against influenza, pneumococcus, meningococcus and Haemophilus influenzae type b) are already recommended in this population [62*]. HIV viremia could temporarily surge through CD4 cell stimulation, but this might be controlled by HAART [57].

As therapeutic vaccines aim to clear present (or latent) HPV infection and HPV-related pre-cancerous and cancerous lesions, they might be more appropriate for PHIV. Therapeutic vaccines against HPV might also remedy the high recurrence rates that are found among PHIV after treatment of intraepithelial lesions. In a mouse model, a therapeutic vaccine against the E7 subunit was more efficacious after removal of most of the tumor tissue [63].

Conclusions

Universal application of the recommendations to vaccinate all girls 9-12 years would eventually protect future HIV-positive women from infection and its cancer sequellae.
However, the protective effect on avoided cancers will only be measurable 20-30 years after vaccination.

An issue of potential importance, in respect to the prevention of HPV-related cancers in PHIV, is whether to vaccinate boys. Decisions are, for the moment, impossible given the lack of evidence on the efficacy of prophylactic HPV vaccines in males. Statistical models predicted however, that if high coverage and persistent protection in females are possible, vaccinating girls only should be sufficient not only to prevent the majority of cervical cancers, but also to ultimately interrupt the circulation of HPV16 and 18 at a population level [64]. This prediction is based on the assumption that the vast majority of HPV transmission worldwide is through heterosexual intercourse and hence is likely to be interrupted by the eradication of infection in one gender only. However, MSM, among whom HIV and HPV infection and anogenital neoplasias are relatively frequent may represent pockets of persistence of HPV transmission not easily interrupted by universal vaccination of girls only.

Given the high cost of the vaccine, special consideration will have to be given to how to distribute it to under-served populations and people in developing countries. As these populations have little access to health care by definition, this will be a particularly challenging aspect of vaccination programs, as is the administration of three injections to young adolescents. Hopefully, if long duration of the efficacy of HPV vaccine would be demonstrated, it could become possible to schedule HPV vaccines in childhood.

HAART is becoming gradually more available in HIV-stricken developing countries. HAART increases the survival of PHIV, but has not been shown to decrease the incidence of cervical and anal cancer [65]. Therefore, at present, special attention should be given to screening and adequate treatment of cervical cancer among HIV-positive women [66*]. The same applies in principle to anal screening in PHIV at high risk of anal cancer [67*].
References

   Using the IARC GLOBOCAN database for the year 2002, this paper estimates that 17.8% of worldwide cancer incidence in 2002 was attributable to infectious agents. *Helicobacter pylori*, human papillomaviruses and Hepatitis B and C viruses were the most important ones. The proportion of infection-attributed cancers is higher in developing countries (26.3%), compared to developed countries (7.7%).


   This meta-analysis update confirms the predominance of HPV16/18 in ICC and HSIL worldwide (prevalence 70% and 52%, respectively) with slight regional differences. The under-representation of HPV16, 18 and 45 in HSIL, compared to ICC, suggests type-specific risks for progression to invasive cancer.

   These recommendations represent the first statement by the Advisory Committee on immunization Practices (ACIP) on the use of a quadrivalent HPV vaccine licenced by the U.S. Food and Drug Administration on June 8, 2006. This report summarizes the Epidemiology of HPV and associated diseases, describes the licenced HPV vaccine and provides recommendations for its use for vaccination among females 9-26 years in the U.S.

   This review focuses on the mechanisms by which HPV can evade recognition by the immune system, in order to persist for decades and cause HPV-related neoplasias.
Although HPV employs several immune escape mechanisms, the immune system successfully clears most HPV infections through innate and adaptive immunity. This article also describes immune interventions in HPV infections and prophylactic vaccines in clinical trials.


This is the first study to show in vitro that HPV 16 E6 and E7 oncoproteins interfere with the activation of the innate immune response through down-regulation of transcription of toll-like receptor 9.


This is the first meta-analysis on published literature from four continents, describing cervical HPV type distribution in women with normal cervixes and pre-
cancerous lesions by region. HPV16 was underrepresented in HIV-positive women, including those with HSIL, compared to the general population. The data on HPV distribution in severe cervical lesions is limited, and almost non-existent for invasive carcinomas.


246
This study is one of the few cohort studies from sub-Saharan Africa to describe that the risk of incident HSIL lesions is more increased in women with high-risk HPV infection and HIV-1 than HIV-2. After adjustment, the risk was primarily associated with HPV persistence, which may be linked to immunosuppression.


This is one of the first studies that assessed clearance of individual HPV types in both HIV positive and HIV negative women. HPV16 and to a lesser extent HPV 18 had lower clearance rates, compared to low risk HPV types. Despite the clear association between HIV serostatus and HPV clearance, the hazard ratio’s for clearance of high-risk types versus low-risk types were not influenced by HIV serostatus.


This is the first record-linkage study on cancer incidence among people with HIV/AIDS in Africa. Increased standard incidence ratio’s (SIR) were found for people with HIV, compared to the general population, for AIDS-defining cancers Kaposi sarcoma (SIR 6.4), non-Hodgkin lymphoma (6.7), and a modestly increased SIR for cervical cancer (2.4).


This is the first cancer registry linkage-study to include direct measures of HAART in adults with AIDS in the U.S. Increased cancer incidence ratio’s (SIR) for non-AIDS defining cancers were observed for anal (SIR 13.4), Hodgkin’s lymphoma (11.5), liver (3.6), oral cavity and pharynx (2.6), respiratory (2.6), leukemia (2.4),...
skin melanoma (2.4), and prostate (1.7) cancers. Effect of HAART on incidence and survival time was not uniform with risk of anal cancer increased after 1995, while anal cancer survival time may have slightly decreased. Underlying mechanisms still need to be elucidated.


This report from one of the largest cases series of vulvar / vaginal and anal intraepithelial neoplasia and vulvar cancers in 241 women from Germany reports very high prevalences of HPV in pre-cancerous lesions and women younger than 56 years with vulvar cancer, compared to women of 56 year or older. Prophylactic vaccines including HPV 16 and 18 could prevent about half of the vulvar carcinoma’s in younger women.


This ongoing follow-up study reports on multicentric lower genital tract neoplasia in 52 women. HPV16 prevalence was particularly high in non-cervical intraepithelial lesions. Multicentric lower genital tract neoplasias seem to evolve through different pathways: high HPV susceptibility and clonal propagation of transformed cervical cell clones.


This study reports higher incidences of non-cervical anogenital intraepithelial lesions in women with HIV, compared to similar cohorts. Increased risk was associated with level of immunosuppression.

This study shows sustained levels of antibodies for at least 5 years against HPV types in the quadrivalent HPV vaccine and 96% efficacy against HPV 6/11/16/18-related persistent infection or disease. No cases of HPV 6-, 11-, 16- or 18- related cervical disease or genital warts were seen in vaccinated women (100% efficacy).


This study demonstrates a sustained efficacy for at least 4.5 years of the bivalent HPV vaccine against HPV16 and -18 endpoints: increased antibody levels (98%), incident infections (96.6%), 12 month persistent infection (100%), HPV16/18 associated CIN lesions and cross-protection against incident HPV 45 and –31 infections.


This is the report of an expert panel by the American Cancer Society, which reviewed existing data on HPV vaccines and formulated recommendations for the prevention of cervical cancer and pre-cancerous lesions through HPV vaccination and cervical cancer screening.


This is the first clinical study to show that young girls and boys (10-15 years) mount HPV 6/11/16/18 antibody geometric mean titres that are non-inferior (1.7 to 2.7-fold higher), compared to young women (16 – 23 years), for which clinical efficacy was demonstrated. More (mild) fevers within 5 days after vaccination were found in the younger group. Otherwise, the vaccine was generally well tolerated.

This one of the few phase II clinical trials in therapeutic vaccines with an MVA E2 recombinant vaccinia virus, injected in the cervix uteri, in patients with CIN2/3. The study demonstrated development of antibody- as well as CTL responses against E2 in all 34 vaccinated subjects. All patients improvement clinically, 20 (59%) showed a total elimination on histology and 11 (34%) had a least 50% reduction in lesion size.


This is the first report from a study on therapeutic vaccine against high grade anal intraepithelial neoplasia (HG-AIN). This phase I trial with HSPE7 in 15 HIV-positive men showed four (27%) lesions regressions to AIN1 and one (7%) to ASC-US. There were also no drug-related or HIV-related serious adverse events, like changes in HIV viral load or CD4/CD8 ratio.


This study in 15 children and young adults with advanced HIV-1 disease demonstrated that 92% of subjects mounted modest, however protective antibody levels after a tetanus toxoid booster. All individuals were on HAART treatment. Shorter booster intervals might be considered in people with HIV.

   This ACIP report has a section on vaccination recommendations for people with altered immunocompetence, including new recommendations about use of live-attenuated vaccines.


   This commentary paper discusses the estimated increase in incidence of cervical cancer in women surviving longer with HIV in countries where antiretroviral treatment has recently become widely available, notably sun-Saharan Africa, and the subsequent need and opportunities for cervical cancer screening.


   This review demonstrates similar test accuracy for anal and cervical pap tests, however, there are insufficient data to conclude on efficacy of pap screening and treatment of precancerous lesions for preventing squamous cell cancer of the anus.
10.4. ANNEX Supporting paper

Performance of acetic acid test when used in field conditions as a screening test for cervical cancer.

Claeys P, De Vuyst H, Gonzalez C, Garcia A, Bello RE, Temmerman M.

Performance of the acetic acid test when used in field conditions as a screening test for cervical cancer

P. Claeys1, H. De Vuyst 1, C. Gonzalez 2, A. Garcia 2, R. E. Bello 3 and M. Temmerman 1

1 International Centre for Reproductive Health, Ghent University, Ghent, Belgium
2 Universidad Nacional Autonoma de Nicaragua, Managua, Nicaragua
3 Servicios Medicos Comunales, San Juan del Sur, Nicaragua

Summary

OBJECTIVE To assess if visual inspection with acetic acid (VIA) is a useful alternative screening test for cervical cancer, when used in a resource-poor setting with an existing cytology-based screening programme.

METHODS Women living in Rivas district (Nicaragua), who attended the programme, were concurrently screened with VIA and Papanicolau (PAP) smear. Screening was performed by health providers who had received training in VIA and a refresher course in cytology. Women testing positive for either of the results were referred for colposcopy and biopsy when indicated. The performance of VIA was compared with PAP smear, calculating the relative true and false positive rate (RELTPR and RELFPR) and for a high threshold on biopsy (cervical intraepithelial neoplasia grade 2 or a higher grade). We determined the trade-off between both tests by calculating the ratio of extra false positives detected through extra true positives (EFP:ETP ratio).

RESULTS A total of 1076 patients were screened. Nearly 33% had a positive screening test. On biopsy, 7.6% had a low-grade intraepithelial lesion, 4.5% a high-grade intraepithelial lesion (HSIL) and 0.5% invasive cancer. The RELTPR (VIA to PAP) was 1.96, the RELFPR 5.02 and the EFP:ETP ratio 8.04. VIA detected twice as much HSIL and invasive cancers as the PAP smear. Yet, for every extra diagnosis, eight extra false positives had to be examined at the referral level.

CONCLUSIONS The VIA spectacularly increases the number of HSIL and invasive cancers detected. The high FPR is a concern for the organization of the referral level. There is a need to establish uniform criteria on test positivity and to further improve the performance in field conditions.

KEYWORDS cervical cancer, screening, visual inspection, cytology, developing countries

Introduction

During the last decade, the problem of cervical cancer has received renewed interest. The decrease in cervical cancer prevalence in most of the developed countries is attributed to the success of cytology-based screening programmes, hardly observed in many developing countries (Sankaranarayanan et al. 2001). The cost and the operational problems related to cytology-based programmes result in the lack of quality screening programmes in resource-poor settings (Parkin 1991; Sankaranarayanan et al. 2001; Sherris et al. 2001). This has attracted the attention of policy makers, health professionals and researchers, and led to the development of alternative approaches to improve the success of screening programmes. One of the new screening tools is visual inspection of the cervix with acetic acid (VIA).

This cheap technique involves the application of 3–5% acetic acid (household vinegar) on the cervix followed by inspection of the cervix 2 min later, under illumination, for the presence of acetowhite areas (Megevand et al. 1996; Sankaranarayanan et al. 1999). A number of studies report test sensitivity for high-grade squamous intraepithelial lesions (HSIL) varying between 70% and 76%, with a specificity of 64.1–79% (University of Zimbabwe/JHPIEGO Cervical Cancer Project 1999; Belinson et al. 2001; Denny et al. 2002). Yet, most of these promising results have been obtained in research settings, with specially trained research staff or health providers performing the test under adequate supervision.

The performance of VIA was desired to be assessed when used in field conditions, particularly as an adequate alternative in a setting where a screening programme based on Papanicolau (PAP) smear already exists.
Visual inspection as a screening test for cervical cancer

Methodology

This study is part of a larger study on integration of cervical cancer screening services in primary health care in the district of Rivas, in southern Nicaragua. The study was approved by the Ethical Board of the Universidad Nacional Autónoma de Nicaragua.

Within this project, women aged 30 years or older who had never been screened, or who had not been screened for the past 3 years (the so-called target population) were invited by community health workers to attend the programme. In line with the national policy, women who attended spontaneously were also screened, irrespective of the time of their last PAP smear. Within this project, the local cytologist responsible for reading all the PAP smears taken in Rivas district within the public health system (2000–4000 annually), received a refresher-training course in July 2000.

In September 2000 and May 2001, seven medical doctors and 26 nurses from six health centres, 13 health posts, one non-governmental organization (NGO) clinic and the gynaecology consultation of the district hospital were trained. The training consisted of 1 day theoretical sessions on clinical and epidemiological aspects of cervical (pre-) cancer, the technique of VIA and a refreshment module on PAP smear sampling. A full day was spent on VIA training, using a pictorial atlas developed at the International Centre for Reproductive Health, Ghent University (not published) and a teaching set of projected 35 mm photographical slides of cervices images after application of acetic acid (cervicograms). Each participant then received 1 day of supervised practical training on women attending the clinics for cervical screening. The trainees received a 1-day refresher workshop 6 months after the initial training, using a teaching slide set and practice sessions.

A positive result on visual inspection was defined as an opaque white or grey lesion with well-defined borders, located close to the squamo-columnar junction. The PAP smears were classified according to the 1991 Bethesda classification (Kurman & Solomon 1994) and considered positive when at least atypical squamous cells of unknown origin were reported. In order to keep the reporting uniform throughout the study period, no adaptation was made to the 2001 Bethesda classification (Solomon et al. 2002).

The two screening tests were performed on all women of the target population attending the health facilities for screening purposes. Other women were screened by PAP smear, with or without VIA test. A PAP smear was obtained using Ayre’s spatula and spray fixative for cytodiagnosis (Laboﬁs; Labonord, Villeneuve d’Ascq, France) and after PAP staining, read by the cytologist of the district hospital in Rivas. All positive PAP smears and 10% of negative smears were revised by a pathologist from the Bertha Calderon Hospital, a referral hospital in Managua. Conventional cytology (dry slides) was used at both levels.

Immediately after the PAP smear, the health providers applied 5% acetic acid to the cervix and recorded the findings 2 min later, using a simple household torch as a light source. Women testing positive on either screening test were referred to the colposcopy clinic. Colposcopies were offered at the NGO clinic in San Juan del Sur, one of the areas of the district. Referred patients were asked to attend the clinic as soon as possible, without previous appointment. The clinic was open every Saturday and colposcopy and outpatient treatment of pre-invasive disease was free of charge.

The referral test involved colposcopy and a biopsy if indicated. Colposcopies and subsequent biopsies were performed by a trained gynaecologist. Biopsies were examined at the Bertha Calderon Hospital by a local pathologist. All biopsies were independently reviewed by a pathologist from Ghent University. This pathologist was blinded for the first result. The overall inter-observer agreement of the biopsies was 66%. Discordant biopsies were investigated by both pathologists, using a binocular training microscope with two heads. The consensus diagnosis was taken as the final result.

Statistical analysis

As only those women testing positive on either VIA or PAP smear were further investigated, hence, introducing veriﬁcation bias, we used speciﬁc statistical methods (Schatzkin et al. 1987; Chock et al. 1997) to assess the accuracy of the VIA compared with the conventional PAP smear as currently used in Nicaragua.

Our data were represented using the sample scheme developed by Schatzkin. As the referral test is not applied to patients who tested negative on both screening test, sensitivity and specificity cannot be calculated. Yet, information about the relative true positive rate (RELTTPR) and the relative false positive rate (RELFPFR) of both the tests is available (Table 1): the RELTPR of test 2 (VIA) compared with test 1 (PAP) = (a + b)/(a + c); the RELFPFR of test 2 (VIA) compared with test 1 (PAP) = (A + B)/(A + C).

We used Mc Nemar’s test, with the usual correction for continuity, to test for a statistically signiﬁcant difference in the sensitivities and specificities between the two tests, even when the sensitivity and the speciﬁcity of the tests cannot be established, as the test compares only the discordant cells within each of the diseased and non-diseased groups:
Table 1 Sample scheme used (Schatzkin et al. 1987)

<table>
<thead>
<tr>
<th>Diseased</th>
<th>Non-diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1+</td>
<td>Test 1−</td>
</tr>
<tr>
<td>Test 2+</td>
<td>a</td>
</tr>
<tr>
<td>Test 2−</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>a + c</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
</tbody>
</table>

Value in parentheses indicates unknown values.

\[
X^2 = (lb - cl - 1)^2/lb + c
\]

We further determined the trade-off between VIA and PAP smear by calculating the ratio of extra false positives (EFPs) to extra true positives (ETPs) detected. According to Chock et al. (1997) this EFP:ETP ratio equals \( (A + B) - (A + C)/[(a + b) - (a + c)] \) and the 95% confidence interval:

\[
CI = \exp[\ln(B - C)/(b - c)] \pm 1.96[(b + c)/(b - c)^2 + (B + C)/(B - C)^2]^{0.5}.\]

The performance of VIA was compared with the PAP test using a high threshold for the referral test: cervical intraepithelial neoplasia (CIN) grade 2 or higher, on biopsy.

The effect of the number of tests done on the test result was assessed in univariate analysis, calculating a \( P \) value for the difference in the false positive rate (FPR) between providers having used the test at least 100 times and the others. The FPR is subject to verification bias as no biopsy was performed on VIA-positive patients with negative colposcopy, but we assume the bias to be equal in both the groups.

Table 2 Distribution of histological results by outcome of screening tests

<table>
<thead>
<tr>
<th>VIA</th>
<th>PAP</th>
<th>N</th>
<th>Did not attend colposcopy</th>
<th>Colpo performed</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Colpo normal</td>
<td>No dysplasia</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>47</td>
<td>4 (8.5%)</td>
<td>43</td>
<td>7</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>275*</td>
<td>52 (18.9%)</td>
<td>223</td>
<td>112</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>30</td>
<td>6 (20.0%)</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>724‡</td>
<td>17 (5.7%)</td>
<td>17‡</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1076</td>
<td>625</td>
<td>307</td>
<td>131</td>
</tr>
</tbody>
</table>

VIA, visual inspection with acetic acid; CIN, cervical intraepithelial lesion; HPV, human papilloma virus.

* Including four patients with no diagnosis on PAP smear because of bad quality.
† Including two patients with no diagnosis on PAP smear because of bad quality.
‡ Patients referred to colposcopies for other reasons, mainly because of presence of polyps.
§ Patients excluded from analysis on comparison of the two tests.
Of 51 patients diagnosed as CIN2 and more on biopsy, 17 were positive on both screening tests, whereas 28 had a positive VIA test only and six a positive PAP smear only. Of 256 patients with a negative referral test, 26 were both PAP and VIA positive, 195 had a positive VIA test only and 18 a positive PAP smear only. The RELTPR of VIA compared with PAP smear was 1.96 (45:23), \( P < 0.001 \). The RELFPR was 5.02 (221:44), \( P < 0.001 \). The EFP:ETP ratio was 8.05 (95% CI: 4.68–13.86).

The FPR of VIA was 82.8%. The FPR decreased with experience: it was 86.8% for health providers who used the test <100 times, compared with 76.8% when used at least 100 times (\( P = 0.04 \)). These rates were similar for nurses and for doctors (Table 3).

Time between screening and diagnosis was significantly shorter for visual inspection than for PAP smear. For 206 patients with a positive VIA and a negative PAP smear, mean time to colposcopy was 17.5 days (95% CI: 14.3–20.8, median 10) compared with 68.9 days (95% CI: 47.5–90.3, median 54) for 23 patients who had a positive PAP but a negative VIA and 36.2 days (95% CI: 24.8–47.5, median 30) for 42 patients with both tests positive (\( P < 0.001 \)).

**Discussion**

Despite the fact that cytology-based screening programmes for cervical cancer have been introduced in most of the countries in South and Central America since the 1970s, they have had very limited success (Sankaranarayanan et al. 2001). Low screening coverage and inappropriate collection and reading of PAP smears and limitations in the accuracy of this test have been shown to be important reasons for the observed ineffectiveness of these programmes (Eluf-Neto & Nascimento 2001).

Our study shows that, despite additional training in correct sampling and reading of PAP smears, the detection rate for dysplasia was only 4.7% in a high-risk population. Earlier data, whereby PAP smears were taken by one single gynaecologist in a general population in Nicaragua, showed a detection rate for abnormal smears of 7.7% (Claeys et al. 2002b). Quality control data revealed that sampling (including lack of endocervical cells and poor fixation) rather than misclassification was the main problem. Yet, compared with the centres where personnel was not trained, the detection rate was three times higher and the number of inadequate samples halved, indicating that the training had an effect on the quality of the PAP smears (data not shown in this paper). Although the performance was improved, the PAP test only detected 47 of 138 lesions, missing nearly half of the HSIL and more than half of the invasive cancers. Conversely, through visual inspection, twice as many pre-malignant lesions of the cervix were detected. This result was obtained after a very short training, without further supervision and by a variety of health providers using the test. This confirms VIA to be a cheap test, easy to perform and with a high sensitivity (Kitchener & Symonds 1999). Our study further showed that the performance increases with experience, as reflected by a decrease in the FPR. The study design does not allow an assessment of the false negative rate, as no gold standard was applied to all people with a negative test. Yet, this might be less important as the main problem of VIA is the low specificity (Wright et al. 2002). Other advantages of visual inspection include the shorter delay in referral and final diagnosis, which is crucial for compliance and timely treatment.

Unfortunately, this does not mean that the ideal screening test for cervical cancer screening has been found. Comparing the test performance of VIA with PAP smear reflects a common situation where one test has a higher true positive rate than the other at the expense of having a higher FPR (Chock et al. 1997). Whereas VIA is known to have a higher sensitivity than PAP smear, its specificity is

**Table 3** Performance of visual inspection in relation to experience

<table>
<thead>
<tr>
<th>Number VIA</th>
<th>VIA result Negative</th>
<th>VIA result Positive</th>
<th>Final result Negative</th>
<th>Final result ≥HSIL</th>
<th>False positive rate Overall</th>
<th>False positive rate Doctors</th>
<th>False positive rate Nurses</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥100</td>
<td>282 (74.0%)</td>
<td>280 (45.0%)</td>
<td>2</td>
<td>99 (26.0%)</td>
<td>76 of 99 (76.8%)</td>
<td>12 of 16 (75.0%)</td>
<td></td>
</tr>
<tr>
<td>5–99</td>
<td>446 (74.7%)</td>
<td>442 (79.7%)</td>
<td>4</td>
<td>151 (25.3%)</td>
<td>131 of 151 (86.8%)</td>
<td>78 of 89 (87.6%)</td>
<td>53 of 62 (85.5%)</td>
</tr>
</tbody>
</table>

VIA, visual inspection with acetic acid; HSIL, high-grade intraepithelial lesion.

* The cases where final results were not available are excluded from the analysis: in groups ≥100: four (14.8%) VIA negative and 23 (84.6%) VIA positive; in groups 5–99: six (15.4%) VIA negative and 33 (84.6%) VIA positive; and 32 VIA carried out by various health providers during training sessions.

© 2003 Blackwell Publishing Ltd
substantially lower. This results in a high number of women needing unnecessary confirmatory investigation. Normal diagnostic procedures consist in colposcopy and biopsy, which are performed at the referral level. The practical impact of this trade-off is shown by the EFP:ETP ratio for VIA compared with PAP smear. This ratio was 8.05, indicating that for every extra case of at least a CIN2 on histology, eight extra false positives had to be attended at the referral level.

The increase in both ETPR and EFPR has a serious impact on the organization of the referral level. In our setting, the detection of more than twice as many lesions through the use of VIA meant quadrupling the number of patients referred to colposcopy and a doubling of the number of patients needing treatment for high-grade lesions or invasive cancer. Using the PAP smear as a primary screening tool, only 77 women would have been referred, 24 low-grade intraepithelial lesion (LSIL) followed-up, 21 HSIL and two cancers treated. In absolute terms, VIA meant a surplus of 245 (of whom 199 attended) referrals to the colposcopy clinic, of 41 extra LSIL needing close follow-up, of 19 extra HSIL and three extra invasive cancers needing specialized treatment and this for 1080 women screened over a period of nearly 2 years.

If visual inspection were to be used as a common screening test, an easily accessible colposcopy clinic would have to be set up at the level of district hospital. Gynaecologists would have to be trained in colposcopy and outpatient treatment modalities, and accept to examine many false positive patients. However, the increase in workload could be countered by focusing the screening programme on older women and increasing the screening interval to 3 years. This would reduce the total number of tests provided and increase the cost-effectiveness of the programme.

Recently, it was shown using a population-based simulation model that VIA, with immediate treatment when abnormalities were found, would be the most cost-effective approach in Thailand if the test was applied at 5-year intervals in women aged 35–55. In the model, treatment consisted of cryotherapy provided at community site and referral for hospital evaluation when a suspected invasive cancer is revealed by the test. However, the authors comment that, depending on resources, test performance and compliance with screening and follow-up, several other options are viable alternatives (Mandelblatt et al. 2002). In Nicaragua, as in most of the Latin American countries, where screening and referral systems, as well as large number of professionals exist, a see and treat approach can hardly be defended. In our study, only 45 of 266 (16.9%) women with a positive VIA test had a lesion that needed immediate treatment (high-grade dysplasia or more). The others would have been unnecessarily overtreated. Moreover, compliance with referral was very good: 82% of referred women attended the colposcopy clinic, which is much higher than the estimated 30% in the previous study. This high compliance rate might have been influenced by the organization of the programme, including the invitation of women of the target population and the provision of diagnosis and treatment free of charge. Yet, health promotion to increase the uptake of a screening programme and the (geographical and financial) accessibility of diagnostic and treatment services should be taken into account in the design of all screening programmes.

From an operational point of view, it is also easier and more feasible to provide treatment at the referral level, than it would be to make cryotherapy available in all primary health centres where screening is currently provided. It might be too early to advocate widespread use of visual inspection as a screening test. Our study, as most of the studies on VIA, focuses on one single test and no information is available on test performance when the test is repeated. It cannot be excluded that the results are positively influenced by the motivation of the health workers who used the test, as half of the nurses did not use it and no information is available on their performance. Yet, these nurses neither performed PAP smears, so most probably they were assigned to other programmes during the study period.

There is an urgent need to establish uniform criteria on test positivity and definitions to evaluate test performance (Denny et al. 2002). Our criteria resulted in nearly 24–25% positive tests (University of Zimbabwe/JHPIEGO Cervical Cancer Project 1999; Belinson et al. 2001; Denny et al. 2002). Using the same criteria in a research setting in Kenya, more than 27% of the tests were positive (H. De Vuyst, personal communication). VIA is a promising test and further field testing to increase its performance is surely needed. Now that more results are available on its effectiveness, standardization and methods for quality control are highly required. Meanwhile, efforts should be targeted into further improving the existing cytology-based programme. Measures to increase coverage (Claeys et al. 2002a) need to be complemented by additional in-depth training of health professionals for correct sampling of PAP smears and further exploring VIA as an alternative screening test.

In conclusion, this study shows that VIA when applied on a larger scale spectacularly increases the number of CIN and invasive cancer detected in a general, but inadequately screened, population. However, the relatively high FPR remains an important concern for the organization of the referral level.
Acknowledgements

The authors thank the health providers of the public health centres in Rivas and of the NGO clinic of Servicios Medicos Comunales whose collaboration made this work possible. This study was supported by the Belgian Development Co-operation through the Flemish Interuniversity Council.

References


Mandelblatt JS, Lawrence WF, Gafflin K et al. (2002) Costs and benefits of different strategies to screen for cervical cancer in less-developed countries. *Journal of the National Cancer Institute* 94, 1469–1483.


Authors

Patricia Claeys, Hugo De Vuyt and Marleen Temmerman (corresponding author), International Centre for Reproductive Health, University Hospital, De Pintelaan 185, P3, 9000 Ghent, Belgium. Tel.: +32 9 240 35 64; Fax: +32 9 240 38 67; E-mail: patricia.claeys@rug.ac.be, icrh@rug.ac.be, hdvuyt@yahoo.co.uk, marleen.temmerman@rug.ac.be

Claire Gonzalez and Alvaro Garcia, Department of Microbiology, Universidad Nacional Autónoma de Nicaragua (UNAN-Managua), AP 663, Managua, Nicaragua. Tel.: +505 277 18 50; Fax: +505 278 67 82; E-mail: osmagar@tmx.com.ni, alvarofidel@hotmail.com

Rosa Elena Bello, SMC, De Enel 30 varas al Este, San Juan del Sur, Nicaragua. Tel.: +505 458 24 02; E-mail: rosabel0@lbw.com.ni
10.5. ANNEX VIA Atlas

This atlas was developed as a training tool and poster aid during VIA examination. The images from the atlas were generated in the PRH Study in Nairobi and the design of the atlas was done by myself. The actual format of the poster is A3 (42 x 30 cm).
Visual Inspection with Acetic Acid (VIA)

A guide to the visual inspection of the uterine cervix

VIA NEGATIVE

- Nulliparous
- Parous, mucus
- IUD strings
- Squamous metaplasia
- Squamous metaplasia
- Atrophy
- Ectropion/erosion
- Discharge
- Polyp
- Nabothian Cyst

VIA POSITIVE

- Acetowhite lesion, opaque
- Acetowhite lesion, opaque, well-defined
- Acetowhite lesion, over columnar epithelium
- Acetowhite lesion, opaque, granish
- Acetowhite lesion, thick, bright white
- Cancer: tumoral mass hemorrhagic
- Cancer: extensive tumoral mass

CRITERIA FOR POSITIVE VIA

- 2 minutes after application of vinegar
- Acetowhite lesions with:
  - COLOR: opaque white, gray
  - BORDERS: well defined
  - LOCATION: on/close to the squamo-columnar junction

Developed by: International Centre for Reproductive Health, Ghent University • Antwerp University • University of Nairobi
Supported by: European Commission-DG Research • Flemish Interuniversity Council (VLIR)