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HUMAN PAPILLOMAVIRUS AS A TARGET FOR CANCER PREVENTION

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Human papillomavirus as a target for cancer prevention

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at ANA Futura Von Behring, 9th floor. Alfred Nobels Allé 8, Karolinska Institutet in Huddinge, the 4th of June 2021 at 13:00

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SAMMANFATTNING

Infektioner som orsak till cancer upptäcktes redan på tidigt 1900-tal. Idag uppskattas det att 20% av alla cancertyper orsakas av infektion. Bland individer med nedsatt immunförsvar, som till exempel organtransplanterade, ökar cancerformer som orsakas av virus, men även cancerformer där det är oklart om infektioner är inblandade ökar. På 80-talet upptäckte Harald zur Hausen att Humant papillomvirus (HPV) orsakar livmoderhalscancer, för vilket han tilldelades Nobelpriset 2008.

Livmoderhalscancer drabbar nästan 550 kvinnor per år i Sverige och årligen dör cirka 190 kvinnor i sjukdomen. Med HPV-vaccin och gynekologiskt cellprov kan sjukdomen upptäckas i tid och liv kan räddas.

HPV är en av de vanligaste sexuellt överförbara sjukdomarna i Sverige. HPV kan ge upphov till cancer 5–10 år efter infektion. Kvinnor blir kallade till gynekologisk cellprovtagning för att söka efter HPV, cellförändringar eller livmoderhalscancer. I Sverige blir kvinnor mellan 23 och 64 års ålder regelbundet kallade till gynekologisk cellprovtagning inom ett organiserat screeningprogram som funnits sedan 60-talet.

Nya metoder med ökad känslighet att upptäcka HPV-relaterad sjukdom kan leda till att fler fall kan behandlas i tid. För alla screeningprogram behövs det mätbara variabler för att kontrollera att programmet håller hög standard. Sjukvården behöver rutinmässigt kunna kvalitetssäkra de nya sätten att analysera cellprover för HPV. Det är viktigt att mäta kvalitén på den nya metoden så att kvinnors hälsa även fortsättningsvis kan garanteras.

I detta arbete användes hälsodataregister och biobanker i Sverige, Norge, Danmark och Island för uppföljning av virus som orsak till cancer hos organtransplanterade patienter, samt för att studera HPV-relaterade sjukdomar efter HPV-vaccination. Vi har även tagit fram förslag på hur vi ska kunna bibehålla god kvalité på gynekologisk cellprovtagning genom granskning av HPV-analyser och registerdata.

I **studie I** följde vi upp individer som hade transplanterat ett organ och såg om de utvecklade en särskild cancertyp. Från resultaten i **studie II och III** föreslår vi en klinisk granskning av HPV-analys och beräkning av nyckeltal i screening. I **studie IV** följde vi kvinnor som flyttat inom Norden och som HPV-vaccinerats för att se om de utvecklade någon HPV-relaterad sjukdom. I **studie V** använde vi en belgisk databas för att undersöka om vi kunde förbättra HPV-analys genom att titta på virusmängd av specifika HPV-typer.

För att kunna eliminera livmoderhalscancer och därmed bidra till att rädda liv, går det att vaccinera pojkar och flickor i skolåldern, samt vid första screeningbesöket vid 23 års ålder och att kvinnorna hörsammar cellprovskallelsen som kommer hem i brevlådan. Det finns goda möjligheter till detta efter den glädjande nyheten att Sveriges regering i april 2021 beslutade om en handlingsplan för att eliminera livmoderhalscancer. Det finns hopp!

ABSTRACT

If we would know more about virus causing cancer, we would have the possibility to prevent the disease. Human Papillomavirus (HPV) causes cervical cancer and is one of the world's most common sexually transmitted diseases (STD). Cervical cancer is a preventable disease, nevertheless, still each year around 550 women are diagnosed, and almost 200 women lose their life to this disease in Sweden.

This thesis aims to present:

- an investigation on the cancer risk among immunosuppressed patients (I)
- suggestions on how to, maintain a high-quality cervical cancer screening programme by annual clinical audits on HPV analysis (II), report quality indicators on cervical screening data (III), and use HPV genotype and viral load data to improve cervical cancer prediction in HPV primary screening (V)
  and,
- how to use registry linkages over the Nordic borders to miminize loss to follow-up (IV).

These population-based studies, and a follow-up study, utilized Nordic national and regional health-data and civil registries and a Belgium database to collect data on immunosuppressed patients, cancer outcomes, and cervical cancer screening and population data.

We identified 43,912 immunosuppressed patients in Denmark and Sweden with 5,709 incident cancers (I). The overall standardized incidence ratio (SIR) varied between 1.6 in long-term dialysis patients in Denmark and 3.5 in the Swedish cohort of solid organ transplanted patients. The largest increase in SIR was observed in non-melanoma skin cancer in the Swedish cohort, 44.7 [*n* 994, 95% CI, 42–47.5].

Routine cytology has a method to estimate sensitivity to identify women diagnosed with cervical intraepithelial neoplasia grade 3 or worse. The HPV primary screening programme in Stockholm used similar method and estimated a sensitivity of 97 % (148/154 women) (II). Key quality indicators in the Swedish cervical screening programme in 2014-2016 presented a 69-70% population screening coverage and 96-97% of women who were followed-up with histology after abnormal cytology within 1 year (III). In a Belgian case-control cohort, including 2,230 LBC samples with HPV genotypes and viral load analysis results, HPV 16 and 18 (>0 copies/µl) and HPV31/33/45/52 (3000 >copies/µl) could predict 87% of invasive cervical cancer within a year. By adding 8 HPV types only 9 additional cases were predicted during a 7 year-period. (V).

A registry-based follow-up study an HPV-vaccination trial used registry searches over the Nordic borders, Denmark, Iceland, Norway, and Sweden to gain completeness.

In conclusion, we identified elevated cancer types in immunosuppressed patients that will need further investigations. We proposed strategies for quality assessment of HPV-analysis

and cervical cancer screening, and how viral load and HPV-genotyping can improve prediction cervical cancer in a primary HPV screening.

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CONTENTS

LIST OF ABBREVIATIONS

AC	Adenocarcinoma
AGC	Atypical Glandular Cells
AIS	Adenocarcinoma *in situ*
AML	Algemeen Medisch Laboratorium database
ASC-US	Atypical squamous cells of undetermined significance
BCC	Basal Cell Carcinoma
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
DK	Denmark
DNA	Deoxyribonucleic acid
EBV	Epstein Barr virus
EMA	European Medicines Agency
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Embedded-Paraffin
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HTLV-1	Human T-cell Lymphotropic Retrovirus type I
HHV	Human herpes virus type 8
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSIL	High grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
ICE	Iceland
KVÅ	Klassifikation av vårdåtgärder
LDP	Long-term Dialysis Patients
LSIL	Low grade squamous intraepithelial lesion
LTFU	Long-term Follow-up
MCC	Merkel Cell Carcinoma

LIST OF ABBREVIATIONS CONT'D

NKCx	Swedish National Cervical Screening Registry
NMSC	Non-melanoma skin cancer
NO	Norway
OPSCC	Oropharyngeal Squamous Cell Carcinoma
ORF	Open Reading Frame
OTR	Solid Organ Transplant Recipients
SCC	Squamous Cell Carcinoma
SE	Sweden
STD	Sexually transmitted disease
VLP	Virus-like-Particles
WHO	World Health Organization

1 INTRODUCTION

Since the early 1900s, scientists have wondered "what causes cancer"? At the time, it was only known that some parasites could cause bladder and liver cancer. It was not until the 1960's that scientists discovered that viruses could cause cancers in animals. Then scientists started to wonder, what if this could be the case for humans as well? The very same decade, a large and costly programme was set up by the US Congress to investigate if viruses could cause tumors in humans. A decade was spent on searching for "tumorgenic viruses", however no conclusive results were found: the scientists were told that they were looking for "rumour viruses" [1]. Today, there are reports confirming that up to 20% of all incident cancers worldwide are caused by infectious agents including viruses, bacteria, parasites and fungi [2].

The primary task of the immune system is to clear present infections by controlling the replication of infectious agents. If the immune system is suppressed, as for organ transplanted patients, the immune system´s control will be impaired which can result in a persistent infection of the infectious agent, leading to an increase of numerous types of microorganism-associated cancers [3]. If we can find a virus that causes a specific cancer, then we can screen for the infectious agent and develop a vaccine and/or treatment to eventually eliminate the disease.

In the Nordic countries, we have a long tradition of keeping population-based health data registries and biobanks with human specimens. With a unique personal identification number, we can link individuals between registries and biobanks. This enables us to conduct sophisticated and powerful longitudinal molecular epidemiological studies, which is one way to identify risk factors associated with cancer [4, 5].

In the 1960's, a major epidemiological study was carried out to look at the cancer mortality among 31,658 nuns in the United States. The conclusion was that the frequency of mortality from cervical cancer was much lower among nuns than in the control group [6]. Nowadays, we know that human papillomavirus (HPV) is a sexually transmitted disease. In the early 80's, Harald zur Hausen established the association between human HPV and cervical cancer [7].

In the early 90's, two groups started to investigate the HPV antigen. The first group set out to express the capsid proteins of HPV [8]. The second group was looking for antigens in the blood and performed experiments on rabbits which led to the finding of a possible pathway for developing a vaccine. With a new technique they could produce "virus-like-particles" (VLPs) [9]. In 2006, the first two vaccines against HPV were introduced [10-13].

Organized cervical cancer screening was introduced in the 1960's in Sweden [14]. At that time, we had no clue about HPV existence, and screening for cancer or precancerous lesions was based on detecting cellular abnormalities in the cervix. The smear test taken at screening was analyzed by a cytologist with a microscope visually searching for any cellular changes. Now that we have known for almost 40 years that HPV is associated with cervical cancer, guidelines have changed, and HPV detection is recommended as the screening strategy. All

levels of such programs should be quality assured, organized, monitored, and evaluated for effectiveness over time [15]. European guidelines are regularly updated and released to dictate quality assurance [16, 17]. Cervical cancer is a preventable and treatable disease and for this reason, WHO has pushed for a call to action to eliminate the disease [18]. This is the first-ever global commitment to eliminate a cancer.

The first part of this thesis seeks to determine if there is any elevated risk of cancer in organ transplanted patients. The second and third studies investigate quality assessment and cervical cancer screening in Sweden. The fourth study is a registry-based follow-up study in an HPV-vaccination trial. The final study investigates if HPV genotype analysis can improve the prediction of invasive cervical cancer.

In summary, the thesis comprises most of the strategies needed (viral association with cancer, screening, vaccination, and prediction analysis) to help eliminate cervical cancer and other HPV-related diseases.

2 BACKGROUND

2.1 INFECTIONS AND CANCER

Already in the early 1900s, there were reports of the parasite liver fluke causing liver cancer and the parasite *Schistosoma haematobium* causing bladder cancer, the two first events in the timeline below (Figure 1) [19].

In 1965, the first virus was linked to human cancer; the Epstein-Barr virus (EBV) was found to be associated with Burkitt's lymphoma [20, 21]. Since then, the EBV has also been linked to nasopharyngeal carcinoma, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type) and Hodgkin's lymphoma [22]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are known to cause hepatocellular carcinoma (HCC) [23, 24]. The discovery of Hepatitis B in 1967 did not suggest a link with cancer and serum antigen could not be produced [25]. However, the discovery led to the first HBV vaccination programme that clearly resulted in a decline of HCC [26]. The next oncogenic virus identified was the human T-cell lymphotropic retrovirus (HTLV-1) which was determined to cause adult T cell leukaemia/lymphoma in 1979 [27]. Later in 1983, Harald zur Hausen examined numerous biopsies from cervix and was able to establish the etiology of cervical cancer and the link between HPV type 16 and 18 and cervical cancer [7].

In 1989, the etiology of Hepatitis C and HCC was established. Human herpes virus type 8 (HHV-8), also called Kaposi sarcoma herpes virus, was discovered in 1994 to be responsible for Kaposi's sarcoma [28]. *Helicobacter pylori* (*H. pylori*) was discovered already in 1979 by Robin Warren in a gastric biopsy [29]. At this time, it was believed that the stomach was entirely sterile, and it was not until 1994 that bacterial infections were added to the International Agency for Research on Cancer (IARC) list of human carcinogens [30]. In 2008, Merkel Cell Virus was discovered to cause a special type of cancer in the skin, Merkel Cell Carcinoma (MCC) [31].

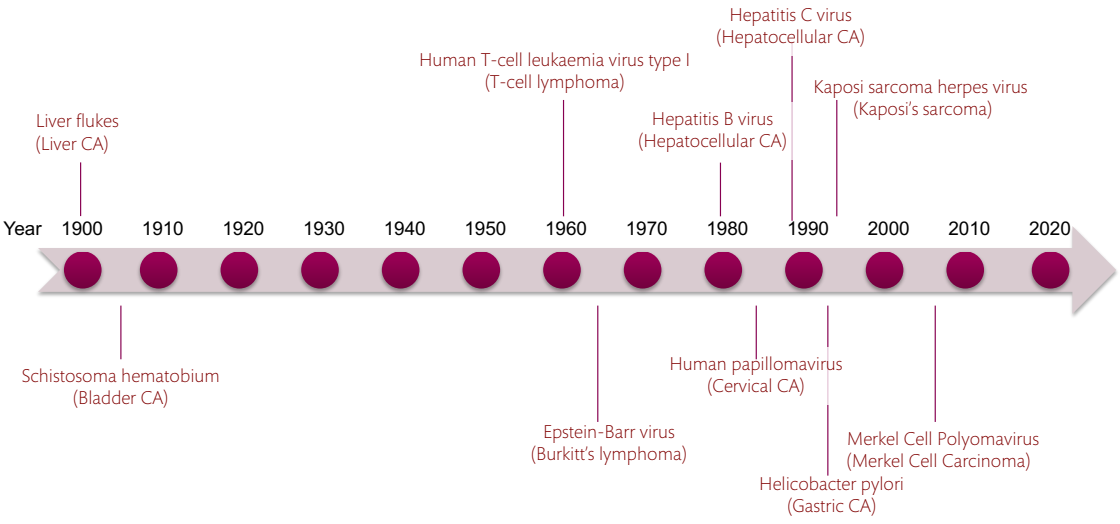

Figure 1. Timeline illustrating the establishment of infections causing cancer.

IARC estimated that 2.2 million cancer cases were attributable to infections in 2018 [32] . The estimates for the proportion of cancers due to viral infections are most likely to be underestimated, as we probably have not found all oncogenic viruses yet. It is difficult to determine the causal role of viruses in the development of cancer for several reasons, as mentioned by zur Hausen in his Nobel lecture:

i) The latency period from time of first infection until cancer development can take 15 to 40 years, with some exceptions.
ii) The infectious agents are not produced in the cancer cells.
iii) Many infectious agents are very common in the population; however, just a small part of the infected individuals will develop the cancer.
iv) The cells will need a mutation in the host-cell genes or within the viral genome to become malignant.
v) Some carcinogens can act as mutagens and facilitate the selection of some specific mutations and act together with the infectious agent [2].

2.2 IMMUNOSUPPRESSION AND CANCER

Immunosuppression among human beings is mainly found due to two reasons: HIV and organ transplantation. Among HIV patients, HPV leads to an impaired lymphocyte function. It is observed that several types of cancer are increased among people living with HIV, especially those that are known to be associated with an infectious agent (Kaposi's sarcoma and Hodgkin's lymphoma, as well as HPV with related diseases[33]). In the western world, the most common reason for a suppressed immune system is a solid organ transplant [34, 35], where the patient receives medication for suppressing his/her immune system so that the new organ will not be rejected.

Some cancers are increased among immunosuppressed patients, suggesting that immunosuppression induces impaired control of tumorigenic viruses [36, 37]. Most of the cancers with an increased incidence after immunosuppression have a known infectious etiology, e.g., HPV and cervical cancer, EBV and Burkitt's lymphoma and HHV-8 and Kaposi's sarcoma [2, 36, 37].

However, some cancers that do not have a known infectious etiology, are also increased among these patients. Compared to other cancers in the general population, non-melanoma skin cancers have the largest increase incidence after immunosuppression: a 65- to 100-fold increase for squamous cell carcinoma [38-40] and a 16-fold increase for basal cell carcinoma [41, 42]. HPV has been commonly found in these tumors - however, the link between the virus and this cancer is not yet established.

Other cancers that have been found to be increased in immunosuppressed patients are cancers of the kidney, thyroid, esophagus, larynx, eye, bladder, lung and colon and endocrine cancers as well as multiple myeloma, leukemia, and melanoma cancer [35, 36]. Increased incidence has

also been seen in patients with long-term dialysis, as these patients are at risk of immunosuppression [43-46].

Harald zur Hausen spoke in his Nobel lecture about how interesting it would be to study cancers that show an increased incidence after immunosuppression, in particular whose etiology is still unknown. Viruses of interest could be new HPV types or polyomaviruses, and cancers of interest then would be glandular cancer and cancers of the eye, thyroid and tongue [2]. Usually, investigations have been only focused on one infection and/or one cancer at the time, which is a slow and tedious process [47]. Modern methodologies in tumor virus epidemiology research are now focused on using Next Generation Sequencing [48, 49] in combination with registry-based research [50]; overcoming the previous slowness. Unbiased sequencing without prior PCR amplification (sequencing everything that is present in a sample) can identify a large number of known and unknown microorganisms that are present in malignant human tissue [51]. Bioinformatically, human sequences may be removed, leaving the metagenome, that is non-human genomes, for study and analysis [52-54].

2.3 HUMAN PAPILLOMAVIRUS

Rabbits infected with papillomavirus were first described in the 1930's [55], and the carcinogenic effects of these viruses in the same animals, a couple of years later [56]. All papillomaviruses belong to the family *Papillomaviridae,* and hosts can comprise mammals, birds, and reptiles [57, 58].

The papillomavirus itself is a small non-enveloped icosahedral virus with a circular double-stranded DNA genome of approximately 7000-8000 bp. The genome consists of three domains, the long control region, responsible for regulating the gene transcription and replication, the early region (E1-E7) involved in viral gene expression, replication, and survival and the late region (L1-L2) which comprises two structural proteins. The human papillomavirus (HPV) may have up to three oncoproteins E5, E6 and E7. Oncoproteins E6 and E7 inhibit the tumor suppressor proteins p53 and pRB (retinoblastoma protein) [19]. Little is known of E5, which is absent in many HPVs, although suggested to play a major role in the interaction with the epidermal growth factor (EGF) and in this way enhance proliferation of infected cells [59].

HPV are classified depending on the similarity of the conserved L1 open reading frame (ORF). HPV isolates belonging to the same genus should have at least 60% sequence identity. Different species within a genus share between 60% and 70% similarity and different types within a species, should share 71-89% of the L1. HPV subtypes and variants should have less than 10% diversity of their DNA isolates [57].

There are up to 222 different HPV types which have been officially established according to the HPV reference center (data accessed April 2021) [60, 61]. 6 HPV types have been withdrawn due to misclassification[62]. Among these, there are 12 HPV types (HPV16, 18, 31, 33, 35 39, 45, 51, 52, 56, 58 and 59) that have been classified as carcinogenic to humans, so called high-

risk types, and one type, HPV68, which is classified as probably carcinogenic, according to the International Agency for Research on Cancer (IARC). All of these 13 high risk types, belong to the genus alpha and are highly oncogenic [19].

The prevalence of HPV varies around the globe. A large meta-analysis estimated the global burden of HPV infections in 2008, including just over a million women with normal cytology results. The highest HPV prevalence was found in Sub-Saharan Africa (24 %), followed by Eastern Europe (21%), this contrasts with the lowest prevalence in North Africa (9%), and Western Asia (2%). In this study, the highest prevalence in Europe was among 25-year-olds (25%) and there was a rapid decline in prevalence in ages above 34 [63]. In a second pooled study including normal cytologies, HPV16 was the most prevalent type in Europe (21%), South America (15%) and Asia (14%), while in Sub-Saharan Africa the most prevalent type was HPV 42 (11%) [64].

2.4 CERVICAL CANCER

As mentioned above, zur Hausen's studies established the etiological link between HPV and cervical cancer and nowadays, we know that the virus is one of the most common sexually transmitted diseases (STD) in the world [65]. Early sexual debut and number of lifetime partners are associated with a higher risk of cervical cancer [66] [67].

Through the cervical stratified epithelium, a microwound is believed to be required, HPV virions can reach the basal cells and establish an infection [68]. Up to 91% of all HPV infections are transient infections and will clear themselves within 24 month[69]. Even though this step is referred to as "clearance", implicating that the infection is not detectable by HPV analysis, it could be that HPV remains in a latent phase.

However, some infections persist for more than 5 years and are then at high risk to progress to cervical intraepithelial lesions of different grades, (low (LSIL) and high (HSIL) grade lesions). LSIL can regress to normal tissue up to 5 years, however, could also persist. HSIL can also regress spontaneously.

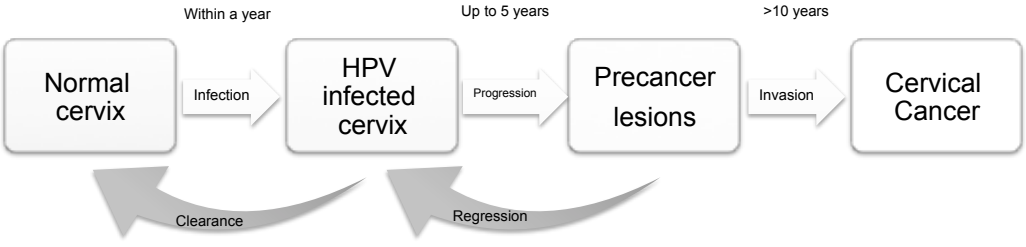

Figure 2. A schematic model of the natural history of HPV (Picture adapted from Schiffman et. al. [70]).

The time for an HSIL to progress into invasive cervical cancer is unknown, and this could be more than 10 years after the infection. Infections with HPV 16 are associated with a more rapid progression than infections with other HPV types. For this reason, HPV 16 infections and associated lesions should be treated upon discovery (Figure 2) [70, 71].

There are two major forms of cervical cancer, the most common one is the squamous cell carcinoma (SCC) arising from the stratified squamous epithelium and the less common one is adenocarcinoma (AC), originating in the glandular epithelium. In a Swedish study including 1,230 identified cervical cancer cases, 74.9% of them were SCC and 19.8% of them were AC [72].

Even though cervical cancer is a preventable disease, it is still the fourth most common cancer among women worldwide, with more than 600,000 cases per year. The estimated age-standardized (world) incidence rate for cervical cancer in 2020 was 13.3/100,000 (published in 2018, Cancer Today database, WHO, data accessed April 2021) [73]. In Sweden, the estimated age-standardized (world) incidence rate was 10.4/100,000 in 2020. The estimated number of deaths due to cervical cancer globally was 341,831 in 2020. The number of deaths in Sweden due to cervical cancer was estimated to 200 in 2020 [73].The estimation for cervical cancer by WHO is somewhat higher than the crude numbers published by the Swedish National Board of Health and Welfare for years 2018 and 2019, where incidence cases of cervical cancer was reported to be 567 and 533, respectively. Death caused by cervical cancer was reported to be 212 women in 2018 and 198 women in 2019 [74].

2.5 CERVICAL CANCER PREVENTION

Already back in the 1920s, long before it was known that HPV caused cervical cancer, Georgios Papanicolaou started with somewhat unconventional methods to study cell samples from the cervix, being the first scientist to find cancer in a cytology sample. It is thanks to him, and his devoted wife Andromachi Mavrogenous, that all women across the world can be screened for precancerous lesions and other diseases related to the female reproductive system[75].

2.5.1 Primary prevention - Vaccination

Any intervention designed to eliminate a disease or injury before it has occurred is called a primary prevention. The vaccine will lower the probability of an individual to acquire a persistent infection with an HPV type included in the vaccine. To date, there are 3 types of prophylactic HPV-vaccines on the market that are approved by the Food and Drug Administration (FDA) [12, 76, 77] and the European Medicines Agency (EMA) [10, 11, 78].

All three vaccines contain VLPs that are assembled from the recombinant capsid protein L1 and include adjuvants that enhance the immune response. In 2006-2007, two vaccines were approved: Cervarix™, a bivalent vaccine protecting against HPV types 16 and 18 developed

by GlaxoSmithKline, and a quadrivalent vaccine, GARDASIL® protecting against HPV types 6, 11, 16, and 18 developed by Merck & Co., inc.

The reason for including these HPV types is that HPV 16 and 18 are responsible for around 70% of cervical cancers cases [79]. HPV 6 and 11 are known to be low-risk HPV-types (not causing cancer, nonetheless these HPV types causes 90% of the genital condylomas[80]).

In 2015, a nonavalent vaccine was introduced, GARDASIL®9, protecting against 9 HPV-types, (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58). This time, they included an additional 5 high-risk types to protect against up to 90% of cervical cancer. An interim with follow-up of eight years confirms effectiveness, no waning below 90% could be seen among 1,448 study participants [81].

EMA and FDA have approved the use of all three vaccines for females and males from 9 years old. The best protection and antibody response will be obtained if the individual is vaccinated before first intercourse. The bivalent vaccine will not protect against condyloma as it does not include protection for HPV 6 and HPV 11. The quadrivalent and nonavalent vaccines will protect against condyloma. All three vaccines protect against cancer and precancerous lesions of cervix, vulva, and anus and other HPV-related diseases (e.g oropharyngeal cancer).

As of October 2020, there were 110 countries (57% of all countries) with a national HPV vaccination immunization programme in place [82]. Since 2006 Sweden had access to HPV-vaccine, however due to procurement issues a school-based programme was not in place until 2012. In the time before the programme was initiated the vaccine was subsidized for girls aged 13-17, later the age was extended to 26 years. All three vaccines are approved for 2-dose schedules, the dose interval for boys is 6 month and for girls 9-13 month [83]. The school-based HPV vaccination programme in Sweden is targeting boys (born in 2009 or later since august 2020) and girls 10-12 years, the nonavalent vaccine is administrated according to the 2-dose-schedule. Sweden has high vaccination coverage, according to The Public Health Agency of Sweden 78% of the boys and 84% of the girls were vaccinated within the programme in the end of 2020 [84].

With the three vaccines on the market, dose and vaccine regimen has been widely debated. A study with a small number of participants (N=31) had their antibody level measured after a mixed dose set-up that included women who had one dose of the quadrivalent and one dose of the nonavalent. The result showed 100% seropositivity with a peak of antibody titers 18-84 month after vaccination, suggesting that it would be sufficient for women with a single dose of the quadravalanet to only receive one dose of the nonavalent for sufficient antibody coverage [85].

With different vaccines marketed overtime and with so many different vaccination programmes ongoing, it is essential to monitor the incidence of low and high-grade lesions

of the cervix, cervical cancer incidence and, if possible, also the antibody response of participants for long-term effectiveness of the vaccines.

A concern has been in regard to the elimination of HPV16/18, will would it be possible that other HPV-types would increase and a type replacement would occur. A large community-randomized study could show no need for concern regarding type replacement. The study had a vaccination coverage of 20-50% for youngsters born 1992-95, all in all 80,000 participants in the cohort. No pattern of type replacement could be found. They did wave for future observation on HPV 51 and 39[86].

The Nordic countries have a suitable infrastructure for performing follow-up studies on monitoring HPV related diseases and for collecting biobank material for HPV analysis [87-89]. Recently, the first study was published proving that the quadrivalent HPV vaccine is effective against invasive cervical cancer. This nationwide study took place in Sweden and used health and population registries to follow more than 1.6 million girls and women ages 10-30 years during 2006-2007. The authors found that there was a clear lower risk of cervical cancer when women were vaccinated with the quadrivalent vaccine. For those who were vaccinated before 17 year of age, 88% had lower risk of cervical cancer, compared to those who never had been vaccinated [90].

2.5.2 Secondary prevention – Screening

Screening, a form of secondary prevention, is applied to a healthy population to identify people at risk of developing a disease as described by Wilson and Jungner in 1968 [91]. They describe 10 principles for early detection of disease:

i) The condition sought should be an important health program.
ii) There should be an accepted treatment for patients with recognizable disease.
iii) Facilities for diagnosis and treatment should be available.
iv) There should be a recognizable latent or early symptomatic stage.
v) There should be a suitable test or examination.
vi) The test should be acceptable to the population.
vii) The natural history of the condition, including development from latent to declared disease, should be adequately understood.
viii) There should be an agreed policy on whom to treat as patients.
ix) The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
x) Case-finding should be a continuing process and not a “once and for all” project.

In the same paper written by Wilson and Jungner, there is a discussion about the existing public attitude to screening and the possibility of cytological self-sampling [91]. There are two types of screening approaches: opportunistic and organized. Opportunistic screening means that it is up to the patient themselves to book a medical appointment for screening. In an

organized screening program, there are regional or national teams who are responsible for sending invitations and coordinating the follow-up of patients [16]. Both types of screenings can be beneficial for the patients [17]. However, organized screening with appropriate follow-up has shown to reduce mortality rates due to cervical cancer by 80% [92].

European guidelines further recommend performing systematic quality assurance of cervical cancer screening. According to these guidelines, cervical screening programs should be organized, population-based, and invitations should be sent at set intervals [16, 17]. All levels of the programme should be quality assured, organized monitoring should be in place and the programme should be evaluated for effectiveness over time [15].

Organized cytological screening was introduced in the 1960's in Sweden [14]. Since 2015, primary HPV screening programme has been recommended instead of cytology for certain ages as defined by the European Guidelines [93] as well as by the Swedish National Board of Health and Welfare [94]. The roll-out of HPV-based screening is ongoing in Sweden.

Nowadays, the screening programme in Sweden is based on personal invitations sent by letter to all resident women [95]. Women are invited to screening between the ages of 23-64. Primary cytology screening is performed every 3rd year for women aged 23-29, followed by primary HPV-screening every 3rd year for women aged 30-49, including a co-testing with HPV-DNA analysis and cytology screening, at age 41, and finally primary HPV-screening every 7th year for women aged 50-64 (Figure 3) [94].

Due to the high HPV prevalence in women aged 23-29, primary cytology is a better option in this age-group. HPV positive samples are reflex tested for cytology. If cytology is positive for cellular abnormalities, it will be followed-up with a colposcopy. Cytology screening in Sweden has reduced the incidence of cervical cancer by 50-75%. However, the result can vary depending on the quality of the sample as well as the cytologist reading the sample [96].

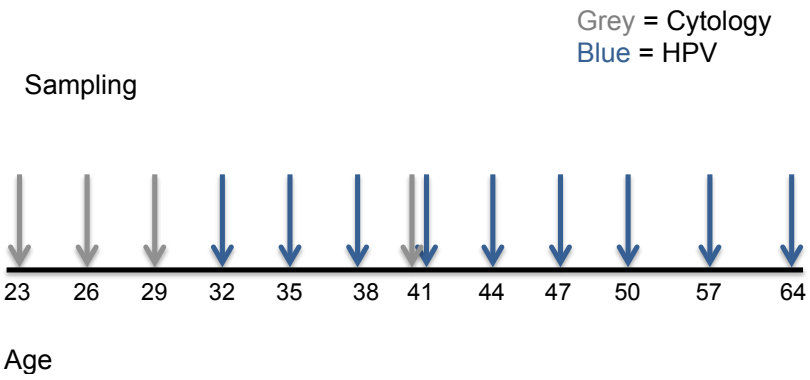

Figure 3. Intervals of cervical screening and methods (grey arrow indicating primary cytology screening and blue arrow indicating primary HPV-screening) [94]. (Picture is adapted with permission from the Swedish National Board of Health and Welfare)

Screening attendance is largely associated with higher relative survival and cure proportion. Women attending the Swedish programme with a detected invasive cervical carcinoma had 92% cure proportion compared to symptomatic women who had 66% [97].

There have been concerns regarding low sensitivity of cytology screening. For this reason, HPV DNA detection is favorable, even though the specificity is lower [98, 99].

A method to evaluate screening performance is to calculate the screening method's sensitivity and specificity. Sensitivity is the probability of a positive test result, given that the individual is diseased, a true positive. If the individual tested positive and was not diseased, then this is a false positive. Specificity is the probability that the test result is negative, given that the individual is disease-free. These measurements of diagnostic tests will evaluate if the method is sufficient to identify diseased and non-diseased individuals [100, 101].

2.6 ELIMINATION OF CERVICAL CANCER

Cervical cancer is a preventable and treatable disease and for this reason WHO is leading the effort to eliminate the disease, the first type of cancer to ever be eliminated [18]. Their key points are:

i) To increase coverage of HPV-vaccination
   ➔ 90% vaccination coverage for girls up to the age of 15
ii) To increase screening coverage
   ➔ 70% screening coverage with advanced tests by age 35 and 45
iii) To treat women diagnosed with pre-cancer and cervical cancer
   ➔ 90% in each group of these women will receive treatment

Australia could be among the first countries to reach elimination of cervical cancer, a recent modelling study illustrated by estimating the age-standard incidence of the disease from year 2015 - 2100. WHO has not defined the incidence rate for elimination, nevertheless 6 cases per 100,000 is considered to be a rare cancer. The model is based on several assumptions, such as Australia will continue to have high vaccination coverage among 15-year-old girls and boys (78.6% and 72.9%, respectively). According to the model, Australia could have reached elimination already in 2028, with only 4 cases per 100,000 [102].

Sweden is also determined to take the lead towards elimination of cervical cancer as it was decided April 2021 to offer all women HPV-vaccination at their first screening visit for the next five to seven years [103].

3 AIMS

3.1 GENERAL AIM

This thesis has used health data and population registries to explore cancer risks in immunosuppressed populations and to determine how to increase cervical cancer prevention by investigating how to improve quality assurance, monitoring, and HPV analysis.

3.2 SPECIFIC STUDY AIMS

STUDY I: To investigate the cancer risk among two immunosuppressed patient groups, organ transplant recipients and patients on long-term dialysis.

STUDY II: To estimate the clinical sensitivity to identify women at risk for CIN 3 or worse by the same method as has been used in cytology-based screening. We aim to have a higher sensitivity for HPV-analysis than the cytology outcome.

STUDY III: To report key quality indicators and basic statistics about cervical screening in Sweden.

STUDY IV: To explore whether loss to follow-up in an international clinical trial could be minimized by multi-country registry linkages.

STUDY V: To assess the HPV-type-specific and viral load-specific longitudinal predictive values for invasive cervical cancer.

4 MATERIALS AND METHODS

4.1 DATA SOURCES

Citizens of the Nordic countries have a unique personal identification number given at birth or immigration. This personal number can be used to link between health data and population registries as well as biobanks within each Nordic country. The Nordic countries have similar population-based nationwide health data registries, (Table1), where it is mandatory to report certain data, however there are country specific differences in laws regarding data sharing, patient information, informed consent, and reporting to authorities.

Table 1. An overview of the Nordic registries used for data retrieval in studies I-IV. In brackets year the registry started followed by the name of registry holder.

REGISTRY	Denmark (DK)	Sweden (SE)	Norway (NO)	Iceland (ICE)	Study I-IV
Population/ Civil registration	(1968-) National board of health	(1968-) Tax office	(1964-) Population registry and Tax administration	(1952-) Icelandic National Registry	IV (DK, SE, NO, & ICE)
Cancer	(1943-) National Board of Health	(1958-) National board of health and welfare	(1952-) Institute of Population-based Cancer Research	(1954-) The Icelandic Cancer Society	I (DK, SE) IV (DK, SE, NO, & ICE)
Hospital	(1976-) National Board of Health	(1963-) National board of health and welfare	N/A	N/A	I (DK, SE)
Death	(1970-) National Board of Health	(1961-) National board of health and welfare and Tax office	(1951-) Norwegian Institute of Public Health	(19171-) The Directorate of Health	I (DK, SE) IV (DK, SE, NO, & ICE
Cervical Screening Results	(1990) Danish Pathology Data Bank	(1997) NKCx and Pathology database	1952-) Institute of Population-based Cancer Research	(1955-) The Icelandic Cancer Society	II, III (SE) IV (DK, SE, NO, & ICE

DK: Denmark, ICE: Iceland; SE: Sweden; NO: Norway.

4.1.1 Swedish National Registry for Cervical Cancer Prevention

The Swedish National Registry for Cervical Cancer Prevention (NKCx) has complete coverage of invitations, cytology, and pathology diagnoses. NKCx is based on exports from the computer systems that send out test results and invitations. Therefore, there is virtually complete coverage of the cervical screening invitations, cytological, histopathological diagnoses, and HPV tests (it is a copy of the actual real-life data) [95].

4.1.2 Swedish National Patient Registry

The Swedish National Patient Registry is kept by National Board of Health and Welfare and was established in 1963. The registry is compiled with data from out-patient visits, day surgeries and hospitalizations. The registry is 99% complete [104]. Examples of information that can be retrieved include discharge diagnoses, date of visit or hospitalizations, clinic identification, and surgical procedures.

4.1.3 Swedish National Cancer Registry

This registry is kept by National Board of Health and Welfare and was established in 1958. All healthcare providers in Sweden must report any new cancers to the registry. Data that must be reported by hospital or laboratory includes clinical and morphological code, laboratory examination, date of visit and individuals that have been diagnosed at autopsy. There are 6 regional cancer centers that do coding and correction work [105]. Completeness is estimated to almost 99% [106]

4.1.4 Swedish National Population Registry

The population registry is kept by the Swedish Tax Agency. Everyone who lives in Sweden for more than one year is in the registry [107]. Some personal data the registry includes are birthplace, immigration and emigration date, address, names, marital status, and date deceased. Sex and date of birth can be read from the 12-digit personal number.

4.1.5 Karolinska University Hospital's Laboratory registry

At the Karolinska University Hospital Laboratory (KUL) in Huddinge, all pathology and cytology diagnosis are entered to their local IT-system, SymPathy (Tieto AB, Malmö, Sweden). The IT-system was updated in 2004, and since then pathology departments at the five regional hospitals in the county of Stockholm enter their pathology data into this system [108]. All topology and histology data from cytology and histopathology diagnoses uses the SNOMED-coding system. For HPV diagnoses, there is SNOMED coding only for HPV-positivity, HPV-negativity, and not sufficient sample. Desirable variables such as sample type (e.g ThinPrep/Surepath), type of HPV-test or biobanking information is not available [109].

4.1.6 The Danish National Hospital Registry

This registry started in early 1976, covers most Danish somatic hospitals, and since 1995, it also covers the out-patient and the emergency units. By covering the entire nation, it is possible to avoid selection bias. The Hospital registry follows the Danish National Board of Health guidelines, on how to collect data, which is updated on a monthly basis and has a nearly complete registration of all hospital events in the country and the accuracy of diagnostic codes is 83% [110].

4.1.7 The Danish Pathology Data Bank

This is a computerized registry that covers all of Denmark and includes all cytology and histology results in the country. Since 1990, all Danish pathology laboratories have used this electronic system and since 1997, there are national guidelines on how to report in a standardized fashion. The registry holds almost 100% coverage of pathology diagnoses (SNOMED coding system). The system continuously error traces to search for missing or incorrect Danish personal numbers and to confirm that all diagnostic statements includes at least one topology and one morphology SNOMED code [111].

4.1.8 The Norwegian Cancer Registry

Directives to report neoplasms and some precancerous lesions to the cancer registry have been mandatory since 1952. The registry collects their data from hospitals, pathological laboratories, general practitioners, and Statistics Norway. Based on data during 2001-2005, the completeness was estimated to be 98.8% [112]. A study assessed completeness for cervical cancer registry data and biobanked paraffin blocks, and determined that the completeness for cervical cancer cases at the registry was 98.6% and the completeness of selected blocks in laboratory was 100% for the years 1985 and 1999 [113].

4.1.9 The Icelandic Cancer Registry

The Icelandic cancer registry was established 1954 and it has been mandatory to report all incident cancer since 2007. Almost all cancer patients in Iceland are morphologically verified by biopsies, and the proportion of verified cases has been estimated to 96.45%. Completeness of the registry is estimated to be 99.15% [114]. The Cancer Detection Clinic initiated nationwide organized screening for cervical cancer in 1964.

4.1.10 Belgian Algemeen Medisch Laboratorium database (AML)

AML has a large database containing HPV results of more than 1.3 million liquid-based cytology samples. AML uses an in-house developed HPV-test analyzing 18 HPV types (HPV 6, 11, 16, 18, 31, 33, 45, 52, 35, 39, 51, 53, 56, 58, 59, 66, 67, and 68). Cervical cytology is classified according to the Bethesda classification system and is performed on all samples prior knowledge of the HPV result. Since June 2006, AML has a serial

co-testing algorithm. The AML HPV database is also linked with the Belgian cancer incidence bank (CIB2014) of the Belgian Cancer Registry.

4.2 DATA EXTRACTION

4.2.1 Study I

The main exposure was solid organ transplantation and cases were identified according to surgical codes for transplantation for all years available until cutoff date of the study in the Swedish National Patient Registry (1963-2011) and the Danish National Hospital Registry (1977-2013). In Denmark they also included subjects who were on long-term dialysis, and the Danish National Hospital Registry was used for this. The transplantation codes used in Sweden for the transplantation were taken from the Swedish Classification of Care Measures (In Swedish "Klassifikation av vårdåtgärder", KVÅ), Table 2. The main outcome of study I is malignant cancer. We received cancer events from 1958 to 2011 in Sweden and 2013 in Denmark, and all cancers were coded in ICD 7.

Table 2. KVÅ-coding used to identify organ transplant recipients registered at the Swedish National Patient Registry.

Organ transplanted	KVÅ-code
Heart and Heart and Lung combined	FQA00, FQA10, FQA20, FQA30, FQA40, FQA96, FQB00, FQB10, FQB20, FQB30, FQB96
Lung	GDG00, GDG03, GDG10, GDG13, GDG30, GDG96
Small intestines, Liver, Islet cells and Pancreas	JFE00, DJ013, JFE96, JJC00, JJC10, JJC20, JJC30, JJC40, DJ005, DJ006, JJC50, JJC60, JLE00, JLE03, JLE10, JLE16, JLE20, JLE30
Kidney	KAS00, KAS10, KAS20

4.2.2 Study II

Exposure were women who had attended the cervical screening in Stockholm county and the endpoint was a histopathological diagnosis of CIN3+ from 1-JAN-2013 to 31-JAN-2014. All patients with a histological diagnosis of CIN3/carcinoma in situ (CIS), AIS or invasive cancer, were obtained from the Karolinska University Hospital's Pathology Laboratory registry, SymPathy.

The SNOMED codes used for identification included: Topology: T83000 and Morphology: M74008 and M8000-M87999. We did to not search any further than the code M87999, as we wanted to exclude sarcomas. If the data searched resulted in more than 300 events, then 300 samples were randomly selected.

To be included in the audit, women had to be histologically diagnosed with CIN3+ and her prior LBC sample should be stored in the cytology biobank. If there was an HPV-

result from the primary screening the result was valid for the analysis. If there was no HPV result, then the LBC sample was retrieved from the Swedish Cytology Biobank and tested for HPV. LBC samples at the Swedish Cytology Biobank are preserved in 96-well microplates (0.75 mL Tracker 2D in Loborack-96w low cover, MPW52337BC3, Nordic Biolabs AB) at -25°C.

4.2.3 Study III

In Sweden, all cervical screening data including cytology, cervical histopathology (26 laboratories) and HPV-test results (28 laboratories) and where applicable, invitations to screening (22 units), are reported to NKCx annually. The collection of data is described in a previous paper [95]. In short, a pre-written data script in SQL, or appropriate language, is used for all reporting laboratories and units to report in similar format. However, diagnosis coding differs between laboratories. At NKCx, all diagnostic codes are translated to the standard nomenclature as recommended by the Swedish Society of Clinical Cytology. Information, regarding new address, migration, and death is collected from the population registry. NKCx is a large analysis database with more than 300 variables. A selection of the most important variables that have been included for this work are described below (Table 3).

Table 3. Example of variables in the NKCx analysis database used for estimation of quality indicator.

VARIABLE	FULL TEXT VARIABLE
AGE	AGE
CANCEL_DATE	DEREGISTRATION DATE – INVITATIONS
COUNTY_ID	COUNTY CODE
HPVDIAG	HPV DIAGNOSE (POS/NEG)
INV_DATE	INVITATION DATE
LAB_ID	LABORATORY ID
PERSON_ID	PERSON-ID (RUNNING NUMBER, DE-IDENTIFIED PERSONAL NUMBER)
REFERRAL_TYPE	REFERALL TYPE
REG_DATE	REGISTRATION DATE (LABORATORY)
REM_CLINIC	REFERALL CLINIC
RESIDC	COUNTY OF RESIDENCE
RESPONSE_DATE	RESPONSE DATE (LABORATORY)
SAMPLE_DATE	SAMPLE DATE
SAMPLE_ID	SAMPLE ID
SAMPLE_NR	PREPERATION ID
SAMPLE_TYPE	TYPE OF SAMPLE
SCR_TYPE	SCREENING TYPE (SCREENING OR OTHER)
SNOMED/LCODES	MORPHOLOGY (SNOMED)
TOPO	TOPOLOGY (SNOMED)
X_SNOMED	SNOMED TRANSLATION ACCORDING TO SWEDISH SOCIETY OF CLINICAL CYTOLOGY

4.2.4 Study IV

This study is a Nordic collaboration involving health data and population registries in Norway, Denmark, Iceland, and Sweden. All women included have been exposed to the quadrivalent HPV-vaccine and are followed-up for any HPV-related disease occurring after vaccination. All participants have signed a consent for passive follow-up from the base study. Prior the cross-border data linkage each country consulted their ethical boards and all countries had approvals to continue the cross-border linkages. Table 1 specifies the different registries that have been used to retrieve data. There are several linkage steps in this study: i) emigration or immigration status from each country's population registry ii) in the event of migration to a collaborating country the subject is followed-up in health data registries iii) if a cervical specimen is sampled in the new country, the specimen is subjected for biobank collection and sent for HPV-analysis in a central laboratory.

4.2.5 Study V

HPV and cytology results were extracted from the AML database, the National Reference Centre for HPV, Antwerp, Belgium, for the period June 2006 - November 2015. The results for 14 HPV types were of interest for our study, including HPV types 16, 18, 31, 33, 45, 52, 35, 39, 51, 56, 58, 59, 66, and 68. The HPV results were expressed in copies/µl and load/µl. Cytology diagnosis were reported according to BETHESDA, (Benign, ASC-US, AGC, L-SIL, H-SIL and AIS).

The AML database contains cancer incidence data from the Belgian Cancer Registry. Selection criteria for the cases were: a valid security number. incidence of cancer (AC, SCC, adenosquamos carcinoma and other malignancies (no ICD-coding obtained)) in cervix uteri (organ code ICD10=C53). HPV analysis data and cytology diagnosis, date of sampling, age at sampling and case control status was delivered for both cases and controls. Furthermore, three variables were included for the controls: type of cancer, age at cancer diagnosis and date for cancer diagnose.

4.3 STUDY DESIGN, POPULATION AND STATISTICAL METHODS

4.3.1 Study I

This population-based registry study included all patients that had a solid organ transplantation (OTR) registered in one of the two National hospital registries (Denmark and Sweden) during the period 1963-2011 in Sweden and 1977-2013 in Denmark. Patients subjected to long-term dialysis (LDP) in Denmark were also included and were identified in the Danish Hospital Registry.

In Sweden we identified 13,429 patients with an OTR and in Denmark 7,375 patients with OTR. Overall, we included 20,804 OTR patients as well as 31, 140 LDP patients and followed-up for any cancer events

Any cancer event before transplantation/dialysis or up to 6 months after the transplantation was excluded from the study. All benign tumors were excluded from the received data file. We used the NORDCAN registry for expected number of cancers in the SIR calculations. Only subjects with diagnosed with ICD-7 coding found in NORDCAN database were included in the study. A case could have more than one incident cancer, thus appearing more than once in the data set.

In the Swedish dataset we identified 2,142 patients who had developed 2,250 incident cancers, and in the Danish OTR dataset, 1,110 patients with 1,286 incident cancers and the LDP dataset included 1,713 patients with 1,873 incident cancers.

Standardized incidence ratios (SIR) compared to the general population were estimated. The NORDCAN registry holds incidence rates by cancer type, sex, gender and 5-years calendar periods. The ratio of observed-to-expected number of cases was expressed as the SIR.

4.3.2 Study II

This is a population–based cohort study including women who attended the cervical screening programme in Stockholm, Sweden, during 2011 and 2012 (Figure 4). All women with histopathologically confirmed CIN3 or cervical cancer (CIN3+) in the following two years (1-Jan-2013 to 31-Jan-2014) were identified in the Karolinska University Hospital's Laboratory registry.

The search resulted in 381 cases that were further searched for their respective specimens in the cytology biobank. It was a prerequisite to have an LBC sample stored in the biobank for HPV analysis before cancer diagnosis. We localized up to 154 LBC samples. Primary HPV screening and cytology results were collected from the database. A total of 63 LBC-samples had been HPV-tested and the rest (91 samples) were retrieved from the cytology biobank to be HPV tested. In the event of obtaining an HPV-negative result in the HPV-primary screening, LBC samples would be re-analyzed together with the biobanked samples.

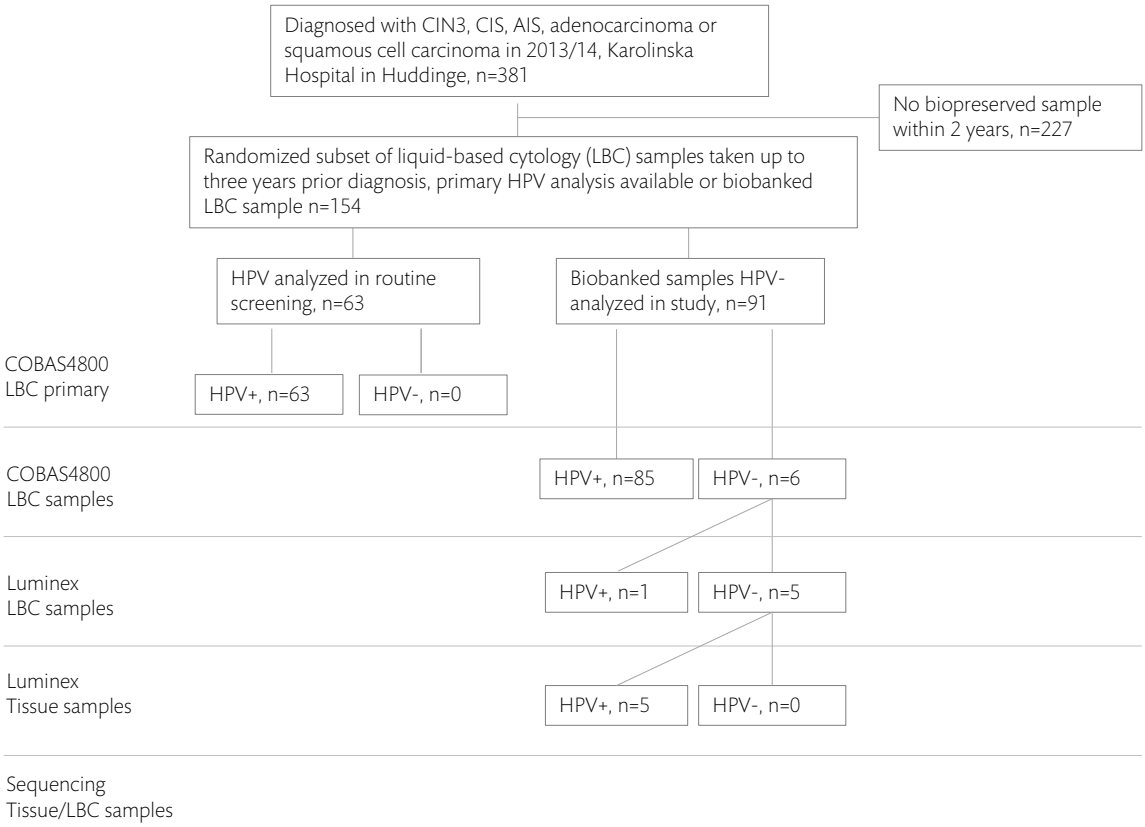

Figure 4. Study II: Study population and sample collection.

LBC: liquid-based cytology

All samples were analyzed by Cobas 4800 (Roche platform), either at primary screening or after biobank retrieval. If any of the samples were HPV-negative, the LBC sample and the subsequent biopsies were analyzed with a broader HPV typing method (modified general GP5+/6+ PCR amplification and Luminex HPV genotyping detection). If the HPV-negativity remained, the LBC sample and the corresponding biopsy were sequenced with the Nextseq 550 system (Illumina, San Diego, CA, USA).

The sensitivity was calculated as the total number of HPV positive detected by Cobas 4800 in the first analysis of HPV primary and biobanked samples, divided by the total number of all identified CIN3+ included in the study. The sensitivity for the cytology-based screening was calculated in the same way, were all cytology diagnosed "Normal" were considered negative. An abnormal cytology is considered positive as the sample would proceed for HPV-testing. The outcome of the two methods were compared by a chi-square test with Yate's correction for continuity.

4.3.3 Study III

The AML database contains cancer incidence data from the Belgian Cancer Registry. Selection criteria for the cases were: a valid security number. incidence of cancer (AC, SCC, adenosquamos carcinoma and other malignancies (no ICD-coding obtained)) in cervix uteri (organ code ICD10=C53). HPV analysis data and cytology diagnosis, date

of sampling, age at sampling and case control status was delivered for both cases and controls. Furthermore, three variables were included for the controls: type of cancer, age at cancer diagnosis and date for cancer diagnose

All cytology and histopathology results are reported using SNOMED-coding. NKCx apply to the SNOMED code system as defined by Swedish Association for Clinical Cytology and Swedish Society of Pathology.

Register-based quality indicators presented are:

- Population test coverage calculates the number of women who had a cervical test the last 3 and 5 years, divided by the total number of women in the same age-group living in the same county, or the whole country for national number, the same period.
- Proportion of organized smears is calculated in two different manners: if the laboratory has a variable indicating it is a sample within the screening programme this number is used in the nominator. Laboratories without this indication then the number for smears sampled by maternity care centres is used as the nominator. The total number of all smears taken is the denominator.
- Attendance rate after invitation within 3 month and within 1 year, was calculated as the inverse of the survival function (1-probability not to participate) by the Kaplan-Meier method.
- The cumulative proportion of HSIL and AIS in cytology that were followed-up with a biopsy anywhere in the country, within 3 month and within 1 year, were calculated as the inverse of the survival function (1-probability of not having a biopsy) by using Kaplan-Meier method.

4.3.4 Study IV

This long-term follow-up study (LTFU) is an extension of a 4-year long base-study. This was a randomized, worldwide (12,167 participants) placebo-controlled double-blind clinical study set up to examine safety, immunogenicity and efficacy of the quadrivalent HPV-vaccine on incidence of HPV 16/18 and HPV related diseases.

In the base-study 5,493 women were from the Nordic countries (Denmark, Iceland, Norway, and Sweden). Upon completion of the study 4,847 women (Denmark 2,046, Iceland 586, Norway 1,463, and Sweden 752) aged 16-23, and who had at least one dose of the quadrivalent HPV-vaccine, consented to the 10-year LTFU study. This study demonstrates how to use registers for cross-border follow-up for consented participants (Figure 5).

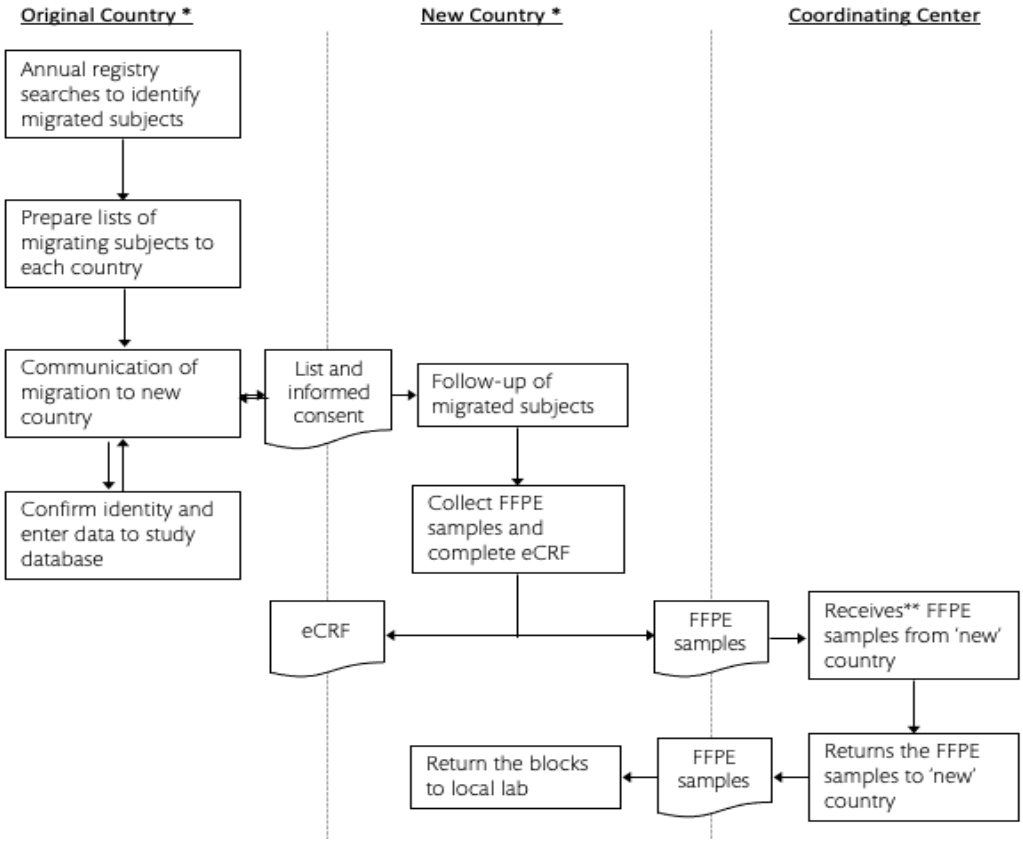

Figure 5. Study IV: Schematic overview of registry linkages for migrated subjects.

* Denmark, Iceland, Norway, and Sweden

** Coordinating center will handle and distribute formalin-fixed embedded-paraffin (FFPE) samples for analysis and reexamination according to protocol, described by Kjaer et. al. [87]

All ethical extensions regarding cross-border linkage were approved as the participants had signed consent of follow-up, however under no circumstances the new study country could contact the migrated participant and no personal identity number could be reported. Copy of the supplementary ethical permissions where shared between the collaborating countries.

Upon ethical approval each country sent the personal identify number to each country's civil registration system, in Sweden the population registry held by the tax office. The search started from the date of last visit (between years 2004 and 2006) in the base study and where to be followed until 1-Mar-2017, here we present data until 1-Mar-2015.

For identified migrated females a list was prepared, and the informed consent was anonymized and shared with the study center in the new country. The Study Centre linked to the country's civil registration system to confirm the immigration and query if a person number were in place. The new personal number was then linked to the cancer registry and the country specific registry for cervical screening results, in Sweden NKCx. Registry data and any, biobank samples identified were collected, anonymized, and sent back to the original country to enter to the study database.

4.3.5 Study V

This population-based longitudinal study extracted HPV-genotype data from the AML database, Antwerp, Belgium. Cases and controls were selected from 946,657 LBC samples with co-testing results (HPV and cytology) between June 2006 and November 2015 (Figure 6).

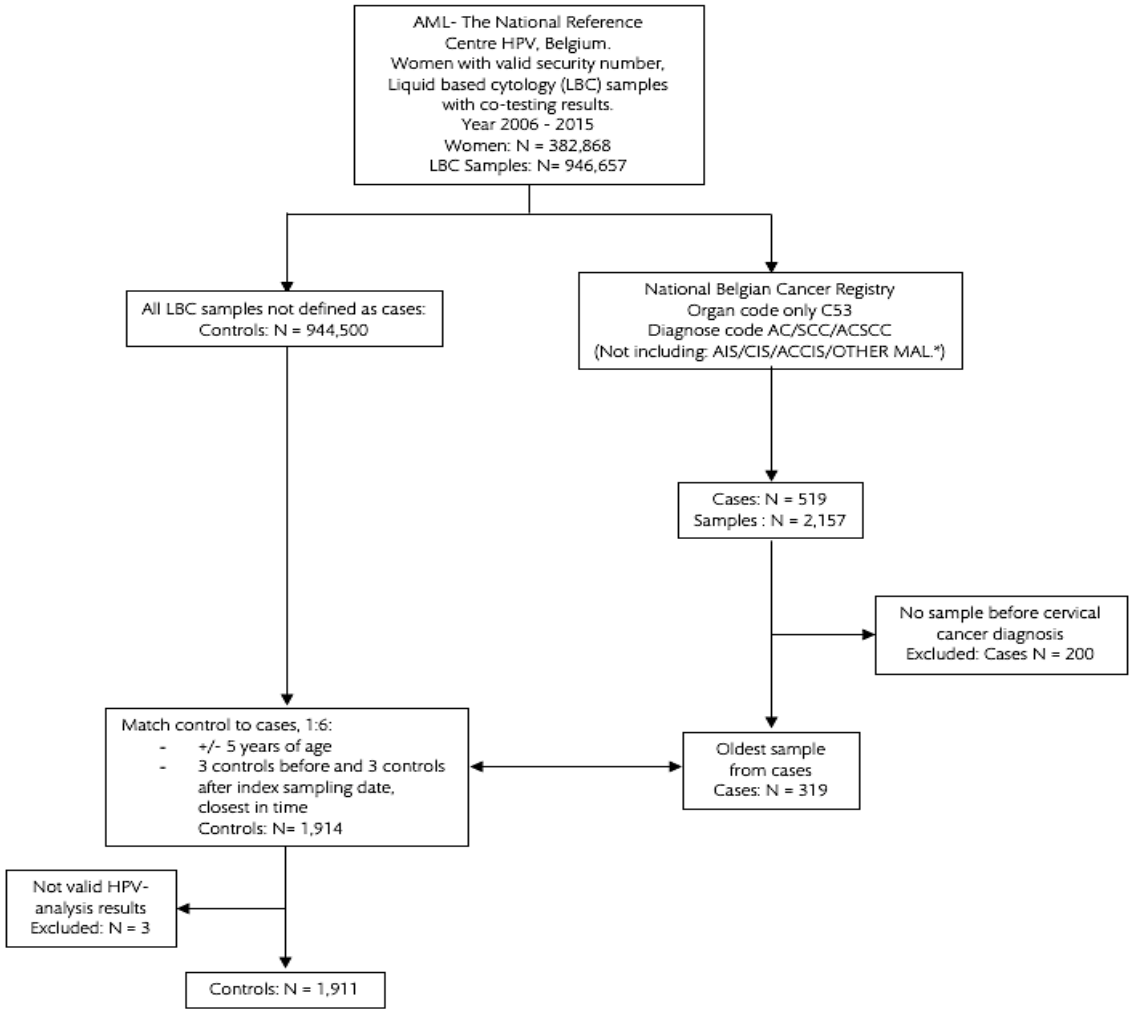

Figure 6. Study V: Data collection.

*AC – Adenocarcinoma, SCC – Squamous cell carcinoma, ACSCC– Adenosquamous carcinoma, CIS – Carcinoma in situ, AIS – Adenocarcinoma in situ, ACCCIS – Adenosquamous carcinoma in situ, OTHER MAL. – Other malignancies

All women included had a valid Belgian security number. Cases were identified with linked data from the National Belgian Cancer Registry with organ code C53 and diagnosed with AC, SCC or adenosquamous carcinoma. Exclusion criteria were diagnosis CIS, AIS and adenosquamous carcinoma in situ. Furthermore, 200 cases were excluded due to no samples before cervical cancer diagnose.

Controls were selected from all women with no history of cervical cancer, which included a total of 944,500 individuals. Cases and controls were matched on a ratio of 1:6, ± 5 years of age and sampled closest in time to the index sampling date, 3 controls

before and 3 controls after. The matching resulted in 1,914 controls; three controls were later excluded due to invalid HPV-results. The study cohort for analysis included 319 cases with 1,911 matched controls.

5 ETHICAL CONSIDERATIONS

All studies, I-V, concern registry data and study II involved HPV analysis of cervical samples. Nevertheless, there were no animal experiments, clinical treatments, or interventions conducted. Data used from the included registries and all sample analysis results are published in aggregated format where no single individual can be identified. Autonomy, self-determination, and integrity must always be respected. Handling of personal data must continuously be assessed to meet the integrity of the study participants. We do this by limiting the number of people who have access to data. Personal numbers are never shared in cross-border linkages and are always encoded. Statistical calculations are performed on an individual level, however only presented in aggregated format.

Consent from each individual was not obtained in studies I-III and study V. Register-based research in Sweden do not need consent. These studies have been approved by the corresponding ethical boards to be conducted without consents (I: DNR 2013/652-31/3, II: DNR 2012/287-31/3, DNR 2012/780-32, III: DNR 2011/1026-31/4, and study V: Belgium Ethical Committee UZA/UA 20/08/086).

Study IV only includes the participants that have consented to be followed-up. An extension of the ethical permission was specifically written, and approved, for the Nordic follow-up (IV: DNR 2008/66 and DNR 2012/547-32).

Data from each individual are collected and treated equally regardless of ethnic background. There is no group that is more or less informed about the research, and all groups, regardless of social class and ethnic background, benefit from research in the future when call-recall systems for screening can be improved and a higher quality of care and treatment may be achieved.

We have not selected any group in particular and we aim to improve the screening programme in Sweden generally and thus benefit all sexually active women who are at risk of cervical cancer. We work with safe methods for preventing privacy intrusion and through our website and annual reports, the public receives information about our research. By gradually reducing the number of cervical cancer cases and improving quality through annual national audits, we consider our research to be more useful than harmful.

In conclusion, the risk of integrity infringement must be considered as low compared to the benefit that this research programme will bring. In the future, women may benefit from improvements in the screening program. This even applies to some of the women who were included in the studies of this thesis.

We believe that the benefit of our studies exceeds the possible risks for each individual

6 MAIN FINDINGS

6.1 CANCER RISK AMONG IMMUNOSUPPRESSED PATIENTS

The final 3 cohorts of OTR and LDP patients from both Denmark and Sweden had a total of 43,912 patients, contributing to 248,672.5 person-years in follow-up (107,988.5 and 60,803 person-years from the Swedish and Danish OTR cohorts, respectively and the LDPs 79,881 person-years).

In Sweden, there were 13,429 OTR's in 1963-2011 and in Denmark there were 7,375 OTR's in 1977-2011. There 1,590 (1,009 in Sweden and 581 in Denmark) patients, who had cancer before organ transplantation and were excluded from the study. The two final cohorts in Sweden and Denmark, of solid OTR's resulted in 12,420 and 6,794 individuals, respectively.

The Swedish cohort had 7,729 men (mean age of 46 years) and 4,691 women (mean age of 45 years). The mean of follow-up was 8.6 years for men and 9 years for the women, starting at the date of transplantation until any type of cancer, emigrated, death or close of study (31-Dec-2011 in Sweden and 31-Dec-2013 in Denmark), whatever event occurred first.

The third cohort identified 31,140 patients undergoing LDP in 1977-2011, with 6,442 individuals excluded as they had been diagnosed with cancer before their first event of dialysis. The final LDP cohort comprised of 15,346 men and 9,352 women, the mean age was 61 years at first dialysis visit. This cohort was observed for a mean of 3.2 years.

All three cohorts were successfully linked to each country's cancer registry. In Sweden, 2,142 individuals in the OTR cohort were diagnosed with 2,550 incidence cancers and in the Danish OTR cohort there were 1,100 individuals who were diagnosed with 1,286 incident cancer. Moreover, in the LDP cohort 1,713 patients developed 1,873 incident cancers.

Several types of cancers revealed the same increase in across the three cohorts. Non-melanoma skin cancer (NMSC), non-Hodgkin's lymphoma and cancers of the lip and oral cavity, kidney, larynx and thyroid are some of the cancers with elevated SIR.

In Sweden the overall SIR among the OTR's was 3.5[*n* 2,142, 95% CI 3.4-3-7) (Table 4). In Denmark the overall SIR in the OTR cohort was 2.9 [*n* 1,110, 95% CI, 2.8–3.1] and in the LDP cohort the overall SIR was 1.6 [*n* 1,713, 95% CI, 1.5–1.6].

The largest increase in SIR was observed in the OTR cohort with patients diagnosed with NMSC, 44.7 [*n* 994, 95% CI, 42–47.5] in Sweden and 41.5 [*n* 445, 95% CI, 37.8–45.5] in Denmark. The SIR for NMSC among LDPs was 5.3 [*n* 304, 95% CI, 4.7–5.9].

In a stratified analysis, the SIR for NMSC among heart and lung recipients was 57.5 [*n* 68, 95% CI, 45.3-72.9] in Sweden and 68.3[*n* 65, CI 95%, 53.5-87.0] in Denmark.

Table 4. SIR of OTR's in Sweden compared to the general population, both sexes

Cancer type	Observed	Expected	SIR
All sites	2,142	605	3.5 (3.4-3.7)
All sites but NMSC, breast, prostate	1,222	429	2.8 (2.7-3)
All sites but NMSC	1,414	638	2.2 (2.1-2.3)
NMSC	994	22	44.7 (42-47.5)
Lip	88	2	41.5 (33.7-51.1)
Lip, oral cavity and pharynx	127	15	8.5 (7.1-10.1)
Non-Hodgkin lymphoma	169	21	7.9 (6.8-9.2)
Salivary glands	10	1	6.8 (3.6-12.6)
Nose, sinuses	7	1	6.3 (3-13.2)
Kidney	100	17	5.8 (4.8-7)
Oral cavity	28	5	5.5 (3.8-8)
Thyroid	24	5	4.9 (3.3-7.3)
Liver	33	8	4 (2.8-5.6
Larynx	12	4	3 (1.7-5.3)
Hodgkin lymphoma	7	2	2.8 (1.3-5.9)
Melanoma of skin	76	32	2.4 (1.9-3)
Colon	107	45	2.4 (2-2.9)
Multiple myeloma	20	9	2.3 (1.5-3.6)
Lung	125	55	2.3 (1.9-2.7)
Soft tissues	9	4	2.2 (1.1-4.1)
Pancreas	34	16	2.2 (1.5-3)
Oropharynx	9	4	2.1 (1.1-4.1)
Stomach	32	15	2.1 (1.5-2.9)

6.2 ESTIMATION OF CLINICAL SENSITIVITY OF HPV ANALYSIS

We identified 154 LBC sampled prior to the histopathology confirmed CIN3+ through registry search, whereof 63 already have had an HPV results and the remaining 91 LBC sampled where retrieved from the cytology biobank.

HPV analysis by the cobas 4800 system had already given HPV positive results for 63 samples within the primary screening program. The samples retrieved from the biobank resulted in 85 HPV positive samples and six negatives. The six negative LBC samples proceeded to be modified by general primer GP5+/6+-PCR and typed by the Luminex method. Only one samples was HPV positive, HPV 33. The corresponding FFPE blocks were retrieved and the block most suitable for extraction was examined and confirmed to be cervical tissue by an experienced pathologist. The FFPE were extracted, modified by GP5+/6+ PCR and typed by the Luminex system. HPV types 31 were found in two blocks and HPV 52, 53 and 67 in three blocks. The six blocks that had been HPV negative were all positive by cytology, and among those, five were diagnosed with CIN3/CIS and one with CIN2.

Our results, 149/154 HPV positive corresponds to a sensitivity of 97%. The cytology results, from the same samples, resulted in 144/154, giving a sensitivity of 94% (Table 5).

The two outcomes from the cytology and virology results were compared by a chi-square test with Yates's correction for continuity. The test demonstrated a statistical difference between the two measures ($\chi^2_{df=1} = 5.01 > 4.03 = \chi^2_{df=1} = 0.05$).

Table 5. Sensitivity of the HPV-analyzes in LBC by cobas 4800 compared to cytology to identify women with subsequent CIN3+ in biopsy.

Method	True Positive	False Negative
cobas® 4800 HPV (HPV types 16, 18 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68)	149/154 (**97%**)	5/154 (3%)
Cytology diagnosis	144/154 (**94%**)	10/154 (6%)

6.3 CERVICAL CANCER SCREENING IN SWEDEN, 2014 - 2016

This study describes the results of all the 2,278,132 cervical smears taken in Sweden at National and County level between 2014 and 2016.

Key quality indicators in Sweden:

- Cervical smears (cytologies and/or HPV tests) in Sweden 2014 – 2016, age 23-60:
  2014: 662,350 **2015**: 695,648 **2016**: 702,946

- Proportion of organized cervical smears in Sweden 2014 – 2016:
  2014: 69% (Lowest in Skåne 55% and highest in Kronoberg 80%)
  2015: 70% (Lowest in Skåne 59% and highest in Jönköping 92%)
  2016: 69% (Lowest in Skåne 53% and highest in Kronoberg 83%)

- Population test coverage according to programme in Sweden 2014 – 2016:
  2014: 81% (Lowest in Kronoberg 71% and highest in Dalarna 91%)
  2015: 82% (Lowest in Kronoberg 69% and highest in Dalarna 91%)
  2016: 82% (Lowest in Kronoberg 70% and highest in Dalarna 92%)

- Attendance rate after invitation in Sweden 2013-15:
  2013 Invited: 415,912 A Smear within 3 months and 1 year: 55% and 68%
  2014 Invited: 429,634 A Smear within 3 months and 1 year: 68% and 77%
  2015 Invited: 467,066 A Smear within 3 months and 1 year: 57% and 70%

- Number of women with HSIL+/AIS in cytology who are followed up with a cervical histopathology. Target: 100% within 3 months.
  2013: 7,982 A histopathology within 3 months and 1 year: 71% and 97%
  2014 : 8,573 A histopathology within 3 months and 1 year: 70% and 96%
  2015: 9,170 A histopathology within 3 months and 1 year: 66% and 96%, (Figure 7)

County	Number of women with HSIL+/AIS in cytology, 2015	Histopathology Follow-Up Within 3 months (%)	Histopathology Follow-Up Within 1 year (%)
Stockholm	1,904	86	97
Uppsala	230	80	97
Södermanland	161	78	96
Östergötland	422	84	99
Jönköping	209	59	96
Kronoberg	137	53	91
Kalmar	270	81	100
Gotland	54	79	90
Blekinge	252	64	97
Skåne	1,382	55	93
Halland	219	70	95
Västra Götaland	1,707	57	96
Värmland	400	66	97
Örebro	297	54	96
Västmanland	185	81	96
Dalarna	178	74	96
Gävleborg	123	72	97
Västernorrland	209	50	97
Jämtland	124	24	99
Västerbotten	469	42	96
Norrbotten	238	70	97
Sweden	9,170	66	96

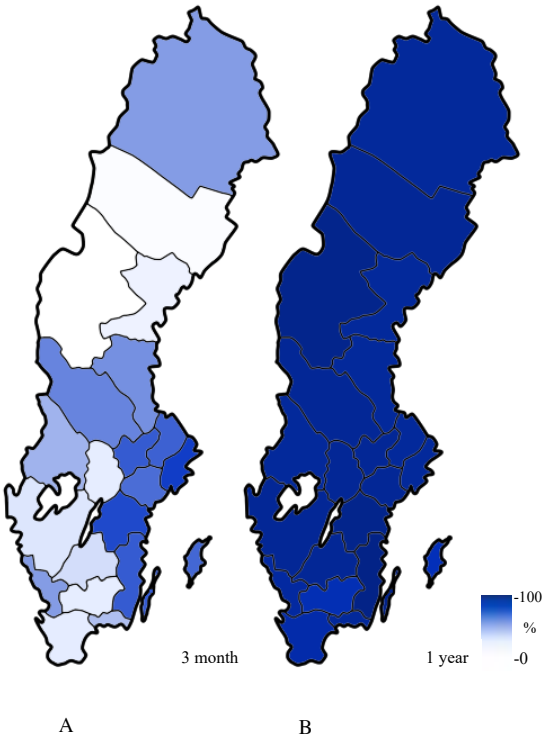

Figure 7. Number of women with HSIL+/AIS in cytology in 2015 and are followed up with a cervical histopathology in (A) 3 month and (B) 1 year.

In SIR data provided by Statistic Sweden for comparison reasons, an increasing trend could be observed for cervical cancer incidence in years 2014-2015 (9.6 per 100,000 in 2014, (between 5.6 to 16.4/100,000 in the different counties), 10.4 per 100,000 in 2015, (between 4.7 to 16.3/100,000 in the different counties) and 11.5 per 100,000 in 2016, (between 9.4 to 17.0/100,000 in the different counties).

More quality indicators of Swedish cervical cancer screenings are presented at https://nkcx.se/.

6.4 CROSS-BORDER LINKAGES TO IMPROVE FOLLOW-UP

We identified 515 study participants that had one or more immigration/emigration event/s to a known or unknown country and 294 study participants had immigrated back to their country of origin, before cut-off date 1-Mar-2015. A person could have several migration events within the study period. We found 273 emigration records (5.6% of all study participants and

58.2% of the 469 first recorded emigrations) out any of the four collaborating study countries and 24 (5.1%) participants had emigrated to an unknown country. Within the 4 Nordic countries, we had 192 women (4% of all study participants and 40.9% of all first migration events) who had both immigration and emigration records. We did not identify any mortalities, or anybody diagnosed with cervical cancer in their Nordic destination country.

6.5 VIRAL LOAD AND GENOTYPE ANALYSIS

In this cohort with HPV and cytology results from 2,230 LBC samples (319 cases with 1,911 matched controls), only two viruses contributed to the prediction of >10% of the invasive cancers, HPV 16 and/or 18 (220/319, 69%), (Table 6).

Among the 1,911 controls 223 were HPV positive (11.7%) for one or more of the 14 HPV types. HPV31/33/45/52 were also frequent in the control group with low viral loads; however, these did not predict any case of cervical cancer (Table 7).

With a HPV method, only analyzing HPV 16/18 at very low detection, as low as >1 copy/µl, with HPV31/33/45/52 at high viral load, >3000 copy/µl, 87% of the cancers can be detected occurring within a year of date of sampling, (Table 8). This algorithm detected 69 % of the cervical cancers even when sampling >3 years prior diagnose, (Table 7). By testing HPV35/39/51/56/58/59/66/69 only predicted 9 more cases in 7 years.

Table 6. Sensitivity at sampling, 0-1, 1-3 and >3 years before diagnosed with invasive cervical cancer.

Time from sampling to diagnosis	HPV 16/18 positive and/or HPV 31/33/45/52 > 3000 copies/µl			HPV 16/18/31/33/45/52/35/39/51/56/58/59/66/68 with copies >0/µl		
	0-1 yr	1-3 yrs	>3 yrs	0-1 yr 2	1-3 yrs 3	>3 yrs 4
True positive	179	39	43	185	42	43
False negative	28	11	19	22	8	19
SUM Truly Diseased	**207**	**50**	**62**	**207**	**50**	**62**
Sensitivity	86.5%	78.0%	69.4%	89.4%	84.0%	69.4%

Table 7. HPV viral load (copies/µl) in cases (A) and controls (B).

A

CASES						
Copies/µl	HPV16	HPV18 (HPV 16neg)	HPV33 (HPV 16/18 neg)	HPV31 (HPV 16/18/ 33 neg)	HPV 45 (HPV16/18/ 33 neg HPV31 <3000 copies/µl)	HPV 52 (HPV16/18/ 33 neg HPV 31/45 < 3000 copies/µl)
1-99	-	3	-	-	-	-
100-999	2	5	-	-	-	-
1000-2999	3	2	-	-	-	-
3000-5999	-	2	-	1	1	-
6000-9999	2	3	1	-	-	1
10000-99999	10	13	2	2	1	-
≥100000	154	21	17	10	1	4
Grand Total	**171**	**49**	**20**	**13**	**3**	**5**

B

CONTROLS						
Copies/µl	HPV16	HPV18 (HPV 16neg)	HPV33 (HPV 16/18 neg)	HPV31 (HPV 16/18/ 33 neg)	HPV 45 (HPV16/18/ 33 neg HPV31 <3000 copies/µl)	HPV 52 (HPV16/18/ 33 neg HPV 31/45 < 3000 copies/µl)
1-99	-	-	-	1	-	-
100-999	7	2	-	10	2	1
1000-2999	7	-	-	4	-	-
3000-5999	2	-	-	2	-	2
6000-9999	1	-	1	-	-	-
10000-99999	12	5	-	6	1	3
≥100000	28	3	8	20	-	10
Grand Total	**57**	**10**	**9**	**43**	**3**	**16**

7 METHODOLOGICAL CONSIDERATIONS

7.1 STUDY DESIGN

Observational studies investigate the natural environment, no interventions are included. Studies I and II are observational population-based cohort studies, study V is a population-based case-control study and study III reports results from the population-based cervical screening register.

It is essential to plan large cohort studies thoroughly before initiation, to determine feasibility of the study. The power calculation prior to study I was based on the number of known transplant recipients, >15,000 new transplantations since 1964 [115], and among these we expected to then identify about 2,000 cancer cases in Sweden. The Nordic countries are a gold mine for exactly these type studies since our health data registries have a very high national coverage and good accuracy. The completeness is often above 98%. That is dependent of the type of disease.

The *registry-based follow-up* study (IV) gives us the opportunity to follow women, that have been a part of a protocol-driven, phase III study, to observe and collect data from their regular health care visits. In the phase III study, these women did not attend the organized screening program, they were asked to go for health visits and per-protocol visits to specific study-centers.

7.2 SELECTION BIAS

Using population registries with high completeness at a national level prevents selection bias due to a minimum of a *loss of follow-up* and *non-response*[116]. Study IV demonstrates a methodology to minimize loss of follow-up by continuing to follow study participants after migration to one of the Nordic countries within the collaboration.

Control selection bias refers to selecting inappropriate controls in a case-control study or a cohort. In study V, we chose controls according to density sampling, and the samples were taken in similar time. We did this by selecting 3 samples closes in time before the index sample and 3 sample after the index sample. The reason why the case and control sample should be sampled approximately the same time is due to that if sampled years apart, routines can have changed regarding sample handling and storage. In our dataset, we had HPV data irrespective of cytology result, which also minimize the selection bias.

7.3 INFORMATION BIAS

Surveillance bias can be seen in groups that are more likely to be followed-up than the general population. A group that is heavily under surveillance, for example cancer survivors, are more likely to be better examined and therefore there is higher chance to find a disease. An overdiagnosis results in an increased incidence of the disease of interest. Surveillance bias can even be seen up to 10 years after initiation[117]. Surveillance bias could be the case in *study*

I where we followed individuals who had a solid organ transplantation or were treated with long-time dialysis. They are more likely to have more health-care visit than the general population, which serves as the comparison group. Regarding overdiagnosis this is unlikely in our dataset. The Nordic countries have a long history of health data registries and have a high-quality system for the diagnosis per se. All incident cancer diagnoses at the Swedish cancer registry must be confirmed by a medical doctor and by histology, a verified diagnose is always more likely to be true.

7.4 CONFOUNDING AND EFFECT MODIFICATION

Confounding is an independent factor that must be associated with the exposure of interest as well as the outcome. The confounder must have an effect and must be an imbalance between the exposure and outcome. A confounder can not be a step in the pathway between exposure and outcome. In register-based studies, the biggest drawback is lack of some of the most common possible confounders including alcohol and smoking. These variables are known to correlate with socio-economic factors (education, salary, and occupation). Our studies (I and III) could have been refined by further registry linkages to collect socio-economic variables that could serve as proxies for alcohol and smoking, as well as diet and physical activity.

Alcohol and *tobacco* are a known risk factors alone and together, and both are strongly associated with cardiovascular disease and several types of cancers [118]. In study I alcohol is associated with liver cirrhosis and other liver and pancreas diseases leading to transplantation and alcohol is also associated with incidence of cancer. We did not adjust for this, as we did not have access to these covariates, alcohol, or tobacco. A model has been proposed by Haldorsen et al., on how to adjust SIR estimations in registry-based studies with these covariates [118]. The algorithm included 14 cancer associated with alcohol and tobacco and then used two models combined for the adjusted SIR in a register-based occupational study.

Age can be a confounder in our studies as HPV prevalence is strongly associated with age, with a very high HPV prevalence in women 20-30 years. In study V we adjusted for this by matching, cases and controls were matched to ± 5 years.

One way of handling confounders could be by stratifying the data. In study I we stratified the different sites of organ transplant, calendar time and 5-years age-groups. Heart and lung recipients had compared to other transplant sites, a significant increased SIR for NMSC, a virus associated cancer. A large nested case-control study with 5,931 organ transplanted patients found an association between immunosuppression drugs and cutaneous SCC [119]. Recently a similar study was conducted in Norway, investigating SCC after solid OTR and also included different immunosuppression regimen. They could see a decline in SCC with time and their conclusion is that immunosuppression drugs have changed from the early 80's until now [120]. It could be of interest investigate if the immunosuppression drugs might act as effect modifier or confounder, we will have to know which drug and for how long time after transplantation it was used. We had the benefit to include long-term dialysis patients (LDP)

in our dataset, who did not receive any immunosuppression medication, instead they have multiple transfusions and are at risk for immunosuppression. The LDP cohort had elevated SIR for similar cancers as in the OTR cohorts in Sweden and Denmark.

7.5 GENERALIZABILITY

Results that could be generalized to other populations or settings are also known as externally valid.

Study I was planned to have a long follow-up time and was set up in two different countries and in two different patient groups. The results showed elevated cancer risks in the different groups in both countries which could indicate that these results can be applied to other populations. Study I was included in a large meta-analysis with 72 prospective cohort studies on cancer risks in solid organ transplant recipients by Huo et. al. The overall cancer risk was similar in the different regions - compared to the general population: OTR had a 2.68-fold cancer risk (SIR 2.68; 2.48–2.89; P <.001)[121].

The method for sensitivity analysis conducted in study II could be applicable to any laboratory performing HPV analysis within an organized screening program. According to Meijer et. al., guidelines for HPV tests in primary cervical screening, the sensitivity for HPV detection should aim to be above 90% [100]. Regarding the results, we aimed to have a higher sensitivity for HPV-analysis than cytology to identify CIN3+. In our study, HPV testing had a sensitivity of 97% while cytology had a sensitivity of 94 %. A recent study comparing cytology and HPV primary test positivity in Finland, Iceland, Norway, and Sweden found that the proportion of HPV primary positive tests were higher than cytology, with a rate ratio of 1.66[122]. Even though this study did not compare true performance, there is an indication of a higher positivity in the HPV primary sampling. However, we could not say that the exact results are generalizable, the sensitivity depend on each laboratory, as the technique, the experience or newly installed equipment could drastically alter the results.

The results from the monitoring of the Swedish cervical prevention programme (III) are very specific for Sweden and the current screening strategy, including screening ages, how to issue reminders and how far each country has come implementing primary HPV screening; these results are not generalizable to other settings/countries, however the methods for calculating are transferrable.

Study V includes more than 1 million HPV and cytology results from more than 380,000 females, with several observations taken during a period of more than three years. This study should be recognized to have results with high generalizability due to the representativeness of the large sample size.

8 DISCUSSION AND IMPLICATIONS

We identified several cancer types with elevated SIR after immunosuppression in Denmark and Sweden (I). NMSC was one of the most elevated with an SIR of 44.7 in the Swedish cohort and could serve some more attention. We proceeded with the Swedish dataset and requested FFPE blocks from local biobanks [123]. In agreement with published studies on NMSCs, we have identified HPV as the most common virus present in NMSCs (present in 95% of total viral reads) and its DNA being present in almost all NMSCs [51, 52, 54, 124-127]. However, RNA sequencing studies reveal that an active HPV infection with transcription is only found in a small minority of skin cancers occurring after solid organ transplantation, suggesting that the huge increase in NMSC after solid organ transplantation is not likely to be attributable to HPV infections[128-130]. Further studies are needed to elucidate the presence of HPVs in skin (e.g., studies on RNA transcription considering pre-cancerous lesions (actinic keratoses and keratoacanthomas) to evaluate possible hit-and-run mechanism) as well as the existence of other viruses.

In the investigation to find viruses that cause cancer, it is important to establish the direction of causality. One of nine criteria should be fulfilled to determine causality between exposure and a possible disease, as set by Bradford Hill [131]. Did the lesion in the tissue attract the infectious agent or does the infectious agent cause the lesion? Study I result could serve as a base for further investigation in the continuing search for virus causing cancers.

In study II, III and V we aimed to increase cervical cancer prevention by proposing annual audit in HPV primary screening, suggestion on how to monitor key quality indicators in organized cervical screening programme and demonstrated how to use specific HPV-types and the viral load in the prediction of cervical cancer in an HPV primary screening program.

The suggested method for audit in an HPV-primary screening setting is already implemented at the Karolinska University Hospital and is conducted every year. Annual audit reports are written each year and reviewed by a senior pathologist and the director of Center for Cervical Cancer Prevention.

The results of monitoring of key quality indicators are presented every year in national reports that are public available. As a part of quality assurance, NKCx also share screening data with the Nordic and Baltic collaboration, NordScreen. Indicators are standardized and cross-country comparison of cancer screening can be accessed via an interactive online tool [132]. This type of collaboration is important, naturally to share expertise in the field, at the same time compare national figures, identify any gaps, or major variations.

In WHO's guidelines on how to assure quality and safety and efficacy of HPV vaccines should include plans for Long-term follow-up commitment[133]. Study IV is a part of such a commitment, and by extending the registry searches cross borders, completeness can be achieved.

HPV primary analysis has higher sensitivity than the cytology, however also a lower specificity, which has resulted in more women that needs follow-up. With a stratification of genotype-specific risk method could reduce theses follow-up colposcopies[134] Sundström et al. confirm in their discussion that HPV genotyping has come to stay and is amor of a question to what extent. They also stress that in mode of new technology, there is a need to keep quality of the cervical screening and the importance of quality assurance and to evaluate effectiveness [135].

Taken together the work within this thesis has prepared the road for further research on elevated cancer types in immunosuppressed patients that could be caused by a virus. We have proposed a method for quality assessment of HPV-analysis and shared data of quality indicators in the Swedish screening program We suggested how to use HPV genotyping and viral load to predict cervical cancer. With a long-term follow-up study cross-borders we can gain completeness in clinical studies where every endpoint can be of importance.

9 CONCLUSIONS

Study I showed increased SIR for specific and similar set of cancer forms among solid organ transplant recipients and long-term dialysis patients in both Denmark and Sweden. Further investigations are necessary to identify any possible etiology.

Study II suggested that the same method that has been used in cytology screening is sufficient to use for audits of the clinical sensitivity to detect CIN3+ in HPV-based screening programs and should continuously be conducted to monitor the performance of the analyses.

Study III showed the monitoring of key quality indicators in the cervical cancer screening programme in Sweden were stable in 2014-2016, however an unexplained cervical cancer increase indicates that more efforts are needed.

Study IV introduced a method for registry-based, follow-up of emigrating and immigrating subjects can be used to increase completeness in international clinical trials in the Nordic countries.

Study V proposed a method how to use specific HPV types and viral load to detect cervical cancer in an HPV-based screening setting. Our result showed it is sufficient to screen for HPV16/18 (0>copies/µl) and HPV 31/33/45/52 (3000>copies/µl).

10 POINTS OF PERSPECTIVE

The elimination era

Today it is exciting times to be a part of the HPV community. Since WHO ‘s call out for elimination of Cervical Cancer initiatives has arisen at EU level (“Europe's Beating Cancer Plan [136]), and at governmental level in Sweden [103].

The vaccination strategies are widely discussed, is there any cross-protection, does immunogenicity differs in sex and ages, or will other HPV related diseases decrease?

Some answers we already have and others we must rely on estimations. There are studies that demonstrates the immunogenicity were it is higher in ages 9-14, compared to ages 15-26 years, while investigating pooled interval data for HPV 16/18 [137]. A metanalysis of the immunogenicity of the quadrivalent vaccine could see higher titer for the girls, regardless of age-group, than the boys [138]. These differences should not affect the chosen vaccination strategy too much.

To reach the target of elimination, which is set to 4 cases per 100,000, gender neutral vaccination strategies are essential, this could be reached even at moderate vaccination coverage. A Finnish randomize community trial, including 33 communities predicted at a gender-neutral coverage of 75%, HPV16 would be eradicated in 30 years [139]. The herd effect has been shown to be slower when girls-only are vaccinated [140].

A meta-analysis including 16 different models predicted herd effects with similar promising results. Assuming an 80% coverage among girls and boys, an elimination of HPV 6/11/16/18 could be expected in 70 years[141]. A Scottish study could see herd protection and cross-protective of the types HPV 16/18/31/33/45, this protection could endures for at least 7 years [142].

Without the HPV-vaccines the global burden of cervical cancers in birth cohorts between 2005 and 2014 could be estimated to be 11.6 million by the year 2094[143]. Further estimations have been carried out looking at the numbers of invasive cervical cancers in the absence of the bivalent vaccine types, HPV 16/18. They used standardized lifetime risk, that decreased from 650 per 100,000 female birth to 157 per 100,00 without HPV 16/18[144].

Not only cervical cancer prevention could gain from this elimination initiative, also other HPV-related diseases. For example, oropharyngeal squamous cell carcinoma (OPSCC)1000 incident cases could be avoided in the Nordic countries, (a population of 27 million) [145].

In Norway an observational registry-based study including HPV related disease at both men and women were used to measure the thought protective/preventable effect of the HPV vaccine. Mostly it will be preventive for the SCC cervical cancers, at the same time they also found to be between 80-90% protective for anal SCC for both men and women, and 76.5% for oropharyngeal cancer at both men and women [146].

It is not only vaccination that is of importance, at the same time women will still need to attend screening. Self-sampling has shown an increased attendance when inviting non-attenders [147]. Several trials are ongoing with self-sampling on urine void [148-151] . This could contribute to an increased screening attendance.

No study alone will eliminate cervical cancer, at the same time we all must do what we can to contribute. This thesis has contributed with suggestion on how the quality in HPV-primary screening can be maintained and given a suggestion on how to improve HPV-primary analysis. The thesis first work paves the way for further investigations on virus as a cause to cancer.

We still continuously must reach for being better, to work out the best vaccination and screening strategies, to improve HPV analysis test with higher sensitivity and specificity and still maintain high quality in all our efforts. We still do not want to miss a sample from a woman that could be at risk to develop cervical cancer. This could be a mother, a sister, or a friend. With all our joint efforts, and with vaccination, screening, and treatment in hand, there is hope that elimination of this disease soon is reality.

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