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THE ARCTIC UNIVERSITY OF NORWAY

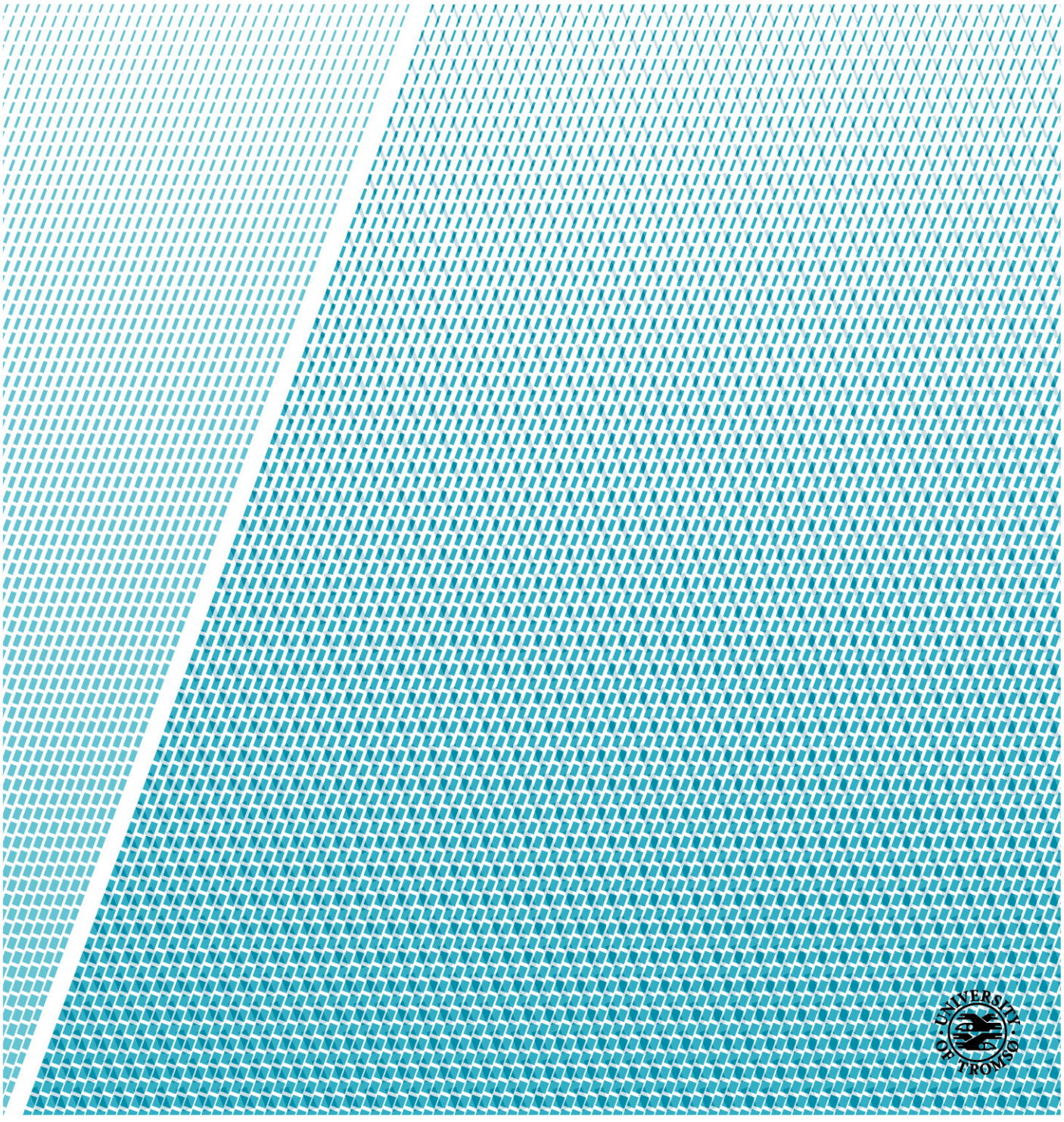
Faculty of Health Sciences

Risk of cervical intraepithelial neoplasia grade 3 or higher (CIN3+) among women with HPV-test in 1990-1992

A retrospective cohort with long-term follow-up of women HPV tested in 1990-1992.

Marit Ø. Riibe

Report: MED-3950 Master thesis/ kull 2015
Supervisors: Sveinung Wergeland Sørbye/Gunnar Skov Simonsen



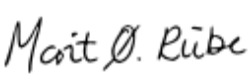
Preface

The project originated in fall 2018 as a part of the module MED-3950 at the medical school at The Arctic University of Norway (UiT) in Tromsø. That fall, our class had just been learning gynecology. From there arose an interest in the HPV virus, which is found to be the main cause of cervical precancerous lesions and cervical cancer. Both of my supervisors had lectures on this topic, one of them as a pathologist, and the other one as a microbiologist. I made contact with Sveinung Wergeland Sørbye at the Department of Clinical Pathology, who agreed to be my supervisor. We discussed possible angles to the topic, and found that it would be interesting to look at unpublished data from HPV testing at The University Hospital of Northern Norway (UNN) in the early 90s, comparing HPV status at baseline with the outcome of cervical intraepithelial neoplasia (CIN3+) in a follow-up period of 28 years. We also wanted to investigate the distribution of four HPV types in CIN3+ and cervical cancer. This became the topic of the Thesis. I also contacted Gunnar Skov Simonsen at the Department of Microbiology and Infection Control at UNN, to co-supervise and to contribute both as a microbiologist and as an employee at UiT.

The writing process

The protocol was submitted before the end of 2018. The following months, all data had to be transferred into an electronic database called SymPathy, using a specific coding system. This made it possible to compare HPV status at baseline with examined outcomes for the same women. The statistical analyses, their interpretation and discussions were done in collaboration with Sørbye. I would like to thank Sørbye for his constructive and pedagogical guidance and his availability throughout the whole process. It has been a true inspiration to collaborate with someone who is so dedicated to his work. I would also like to thank my co-supervisor Simonsen for thorough proofreading and sharing his knowledge on technical requirements for a Master Thesis. The project received no financial support.

August 13th, 2020



Marit Østlyngen Riibe

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Abstract

Background/objective: Long-term follow-up of patients with positive tests for Human Papilloma Viruses (HPV) is rarely studied. The study objective was to compare HPV status at baseline with the outcome of CIN3+ in the follow-up period of 28 years.

Materials and methods: All women referred to the HPV outpatient clinic at the University Hospital of Northern Norway (UNN) in 1990-1992, having a HPV test performed during that time, were included in this retrospective cohort. An exposed cohort with positive high-risk (HR) HPV test was compared to a control cohort with negative HR-HPV test. Both cohorts were followed up to the last time-point of observation of 28 years.

Results: The risk of CIN2+ among HPV positive and HPV negative women was 57.0% (127/223) and 17.4% (73/419), respectively ($p<0.01$). Among the 223 HR-HPV positive women, 102 (45.7%) developed CIN3+, while 44 (10.5%) out of 419 HPV negative women developed CIN3+ ($p<0.01$). The overall cumulative incidence of CIN3+ was 22.7%. Women with HPV33 had the highest incidence of CIN3+, but HPV16 provided the greatest long-term risk of CIN3+. The incidence of CIN3+ was similar for women with a negative HR-HPV test and women with a positive low-risk (LR) HPV test, including HPV 6 and 11.

Conclusion and consequences: HPV status at baseline is predictive for women´s subsequent risk of developing CIN3+. Women with a positive HR-HPV test in 1990-1992 had a significantly higher risk of CIN3+ during 28 years of follow-up compared to HR-HPV negative women. The cumulative incidence of CIN3+ within the two groups were quite similar to that of CIN2+, suggesting that most women with prevalent CIN2+ also developed CIN3+. Our results suggests that detection of LR-HPV types does not predict CIN3+ and therefore should be omitted from primary screening for cervical cancer.

1 Introduction

1.1 Cervical cancer

Cancer of the cervix uteri ranks fourth of cancer types for both incidence and mortality among women worldwide (1). It develops over several years through a series of precancerous lesions, also called cervical intraepithelial neoplasia (CIN) (2). The endocervical canal is covered by columnar epithelium, while the vaginal canal is covered by squamous cell epithelium. 85% of cervical cancer occurs in the squamous columnar junction, which mainly consists of a thin metaplastic epithelium. The Norwegian Cervical Cancer Screening Program (NCCSP) has since 1995 recommended primary screening by cervical cytology for women aged 25-69 years (3). In 2015 HPV testing in primary screening was started as a pilot project in four Norwegian counties, including women aged 34-69 years. It has been decided, due to the strong aetiological association between cervical cancer and HPV, that HPV tests should be introduced in all Norwegian counties during 2019-2022.

1.2 Human Papillomavirus and cervical cancer

Infections with high-risk HPV are the main cause of cervical intraepithelial neoplasia and cancer. 96% of cervical cancers are attributable to one of the 13 most common HPV types in cervical cancer (HPV16, HPV18, HPV45, HPV33, HPV31, HPV52, HPV58, HPV35, HPV39, HPV51, HPV59, HPV68 and HPV56). Addition of seven more HPV types will increase the proportion by 2.6%, to reach a total of 98.7% of all HPV positive cervical cancers (4). Persistent HPV infection is the most important risk factor for cervical cancer. Although HPV infection is the necessary cause for cervical cancer, it is very common and usually transient (5, 6). Approximately 80% of sexually active women acquire an HPV infection during their lifetime. A study by Moscicki et al. estimated that ~70% of women were found to have HPV regression within 24 months, and that women with low-risk HPV type infections were more likely to show HPV regression than women with high-risk HPV type infections (7). Even most of infections that causes cervical intraepithelial neoplasia regress due to an immune response.

Time from HPV-infection to detection of CIN and cervical cancer is well studied. One study suggests that detection of CIN3 occurs 9.4 years after HPV infection (8). Another study reveals that development from CIN3 to cervical cancer takes 10-20 years, depending on genotype (9). Figure 1 illustrates the correlation between HPV infection, CIN and cervical cancer. The occurrence of high-risk HPV infection is highest after sexual debut and typically frequent partner change (15-29 years). The occurrence of CIN2+ is highest 10 years later. The occurrence of cervical cancer is highest in women aged 35-40 years. This knowledge forms the foundation for vaccination and screening to prevent and discover precancerous lesions.

1.3 Vaccination

Given the strong aetiological association between high-risk HPV infection and cervical cancer, the Norwegian Ministry of Health and Care Services decided that as of 2009, all Norwegian 12-year old girls should be offered a vaccine that protects against the most frequent human papillomaviruses associated with cervical cancer (HPV type 16 and 18). Catch-up vaccination of women born 1991-1996 has also been done to protect against cervical cancer. Today, the HPV vaccine offered for free in the childhood vaccination program, is Cervarix. The same vaccine was used in catch-up vaccination of women that were not adequately vaccinated, an offer that lasted from November 2016 until June 2019. Cervarix protects against the two most important HPV viruses, HPV16 and HPV18, which causes 70% of all cervical cancer (4). Another HPV vaccine, Gardasil 9, protects against the seven most important HPV-viruses (16, 18, 45, 33, 31, 52, 58), causing 90% of cervical cancer, in addition to low-risk HPV types 6 and 11 protecting against condyloma acuminata (genital warts). As of 2017, Gardasil 9 is available through Norwegian pharmacies.

1.4 Interpretation of cytology and histology

“Bethesda System 2014” (10) is the system used for classification of cervical cytology, and it is the World Health Organization (WHO) classification for histology. All women aged 25-69 receive a reminder every third year to take a pap smear (cytological sample from the cervix).

If the pap smear is normal, the risk of cervical cancer is low, and the woman is recommended to wait three years before her next pap smear. Cellular changes in cytology is graded as atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (LSIL), atypical squamous cells – cannot exclude high-grade lesions (ASC-H), and high-grade squamous intraepithelial lesion (HSIL). Cytological changes must be confirmed by biopsy before any treatment. If cytology shows high-grade cytology (ASC-H or HSIL) the woman is referred to a gynaecologist for colposcopy and cervical biopsy. The same is the case for women with low-grade cytology (ASC-US or LSIL) if the HPV test is positive for HPV type 16 or 18. Women with low-grade cytology and other HPV types are recommended repeat HPV testing after 12 months. Women with two positive HPV tests are recommended colposcopy and biopsy. Histologically confirmed precancerous lesions are graded as CIN1 (low-grade dysplasia), CIN2 (moderate dysplasia), CIN3 (severe dysplasia) and cervical carcinoma. Women with high-grade lesions (CIN2+) are usual recommended treatment by conisation (LEEP) to avoid progression to cervical cancer (11). According to the annual report of the Norwegian Cervical Program, in 2016, 6489 women were treated for premalignant lesions by conisation. At the same time, mean age at time of conisation was 36.8 years (median 34 years) (12).

1.5 New guidelines including HPV-test

Several studies have documented that tests for HPV have higher sensitivity than cytology alone in detecting high-grade cervical intraepithelial lesions (CIN2+) (13-17). A study by Rebolj et al. suggested that baseline HR-HPV testing and early recall required approximately 80% more colposcopies, but detected substantially more CIN than liquid based cytology alone (18). The HPV-DNA test will be included as a part of the Norwegian cervical screening program during 2019-2021, but only for women 34-69 years of age. Because of the high prevalence of HPV infections in the youngest screening population, HPV test is not recommended as primary screening in this group in order to avoid the risk of unnecessary examinations and treatment. Cervical cancer screening now includes cervical cytology every 3rd year for women 25-33 years of age, and primary HPV testing for women 34-69 years of age. The very low incidence of CIN3+ three years after negative HPV test supports extension

of the screening interval from three to five years (18). In Northern Norway cervical samples are routinely collected by general practitioners and gynaecologists using the ThinPrep liquid-based cytology system. All cervical and histological samples collected in Northern Norway are analysed at the Department of Pathology at UNN Tromsø and at Nordland Hospital in Bodø. The results from cytology and/or HPV testing decide further follow-up of the patients.

1.6 HPV mRNA as an improvement of the Screening Program

Due to the low test-sensitivity, cervical cytology misses 60-70% of all cervical cancer (12). Even though combined HPV-DNA and cytology has higher sensitivity in detecting high-grade cervical intraepithelial lesions (CIN2+) than cytology alone (19), a test with higher specificity and higher positive predictive value is much needed for quality assurance of cervical cytology and to avoid unnecessary treatment. Young women often have positive HPV DNA tests. Several young women also have CIN2 which recedes by itself without treatment. The risk of CIN3 is also high in young women, but they have low risk of cancer caused by other HPV-types than 16, 18 and 45 (9). An HPV mRNA test detects the overexpression of the viral oncogenes E6 and E7 of the three main high-risk human papillomaviruses, namely 16, 18 and 45, which are associated with 86% of cervical cancer in Europe (20, 21). This mRNA-test is more specific for CIN2+ than a test that detects the presence of viral DNA (20-22). Since April 2016, the University Hospital in Northern Norway (UNN) has been testing more than 100 000 cytology samples that originally had been interpreted as normal. The results so far indicate that concomitant testing with cervical cytology and 3-type HPV-mRNA test, particularly in younger women, detects more CIN2+ and can prevent more cases of cervical cancer than cytology alone (23).

1.7 HPV-DNA testing in Northern Norway 1990-1993

In 1990, an HPV outpatient clinic was established at UNN Tromsø as a collaboration between specialists in different departments including gynaecologists, pathologists and microbiologists. The HPV test used detected four of the five most commonly high-risk HPV types including HPV16, HPV18, HPV31 and HPV33. The test also detected the low-risk human papillomaviruses 6 and 11, which are associated with benign condylomatous lesions (genital

warts).

1.8 Relevant literature

A study from Denmark concluded that HPV types 16, 18, 31 and 33 are the most common HPV types in CIN3+ (5). The same study, by Kjaer et al., estimated the long-term risk of high-grade CIN after one-time detection of high-risk HPV DNA and after persistent infection with individual high-risk HPV types. A cohort of 8656 women from the general Danish population was examined twice, two years apart, and had swabs taken for HPV DNA analysis. For women with normal cytology who were concurrently HPV16 DNA positive at the second examination, the estimated risk of developing CIN3+ within 12 years of follow-up was 26.7% (95% confidence interval (CI) = 21.1% to 31.8%). The corresponding risks among those infected with HPV18 was 19.1% (95% CI = 10.4% to 27.3%), with HPV31 14.3% (95% CI = 9.1% to 19.4%), and with HPV 33 14.9% (95% CI = 7.9% to 21.1%). By contrast, the risk of CIN3+ following a negative HPV DNA test was 3.0% (95% CI = 2.5% to 3.5%) (5).

Another study, by Khan and Castle et al. (24) discovered that the 10-years cumulative incidence rates of CIN3+ were 17.2% (95% CI = 11.5% to 22.9%) among HPV16 positive women and 13.6% (95% CI = 3.6% to 23.7%) among HPV18 positive women, but only 3.0% (95% CI = 1.9% to 4.2%) among women positive for other HPV-type viruses. Castle et al. have published yet another study (25) to describe the long-term (> 10 years) benefits of clinical HPV-DNA testing for cervical precancer and cancer risk prediction. They found that a baseline negative HPV test provided greater reassurance against CIN3+ over the 18 years of follow-up than a normal pap smear. This findings was supported by Dillner et al. who described a cumulative incidence rate of CIN3+ after six years of follow-up to be considerably lower among women negative for HPV at baseline (0.27%) than among women with normal cytology (0.97%) (15). A 2019 study by Sand et al. (26) concluded that persistent HPV16 infection was associated with the highest risk of CIN3+, with an 8-year absolute risk of 55% (95% CI = 45% to 66%), followed by HPV33 with 33% (95% CI = 20% to 50%), HPV18 32% (95% CI = 20% to 48%) and HPV31 31% (95% CI = 21% to 46%).

1.9 Study objective

Long-term follow-up of patients with positive HPV tests is insufficiently studied, and our follow-up period of 28 years is relatively unique. It has previously been shown that a single positive test for high-risk HPV in a woman with normal cytology is predictive of her subsequent risk for developing high-grade cervical intraepithelial neoplasia (24, 25, 27). The aim of this retrospective registry-based cohort study was to compare HPV status at baseline with the outcome of CIN3+ in the follow-up period. The study will investigate the distribution of four HPV-types in high-grade cervical intraepithelial neoplasia (CIN3+) and cervical cancer. Because we only have one HPV test at baseline, we cannot for sure know anything about persistence of HPV infections. Regardless, we can assume that women with detected CIN3+ have had persistence of HPV over many years. Differences between the two groups, regarding cumulative incidence of CIN3+, for both HPV positive and HPV negative patients, will be described in this cohort.

2 Materials and Methods

2.1 Study design and data

This study is a retrospective registry-based cohort with a prospective design. The datacollection consists of cytological and histological diagnoses from the Norwegian Cervical Cancer Screening Program among the population in Troms and Finnmark counties with an HPV-DNA test at the HPV outpatient clinic at UNN in 1990-1992. The first HPV test is registered from 1st of July 1990. The last HPV test is registered in a pap smear from October 15th 1992. Our histological follow-up lasted until 31st of December 2018. The diagnoses was retrieved from the diagnostic database SymPathy at the Department of Clinical Pathology, which receives and analyses samples from the screening program.

2.2 Exposed cohort and control cohort

An exposed cohort consisting of women with a positive high-risk HPV test (N=223) was compared to a control cohort consisting of women with a negative high-risk HPV test (N=419). Both cohorts were followed up according to the national guidelines in the Norwegian Cervical Screening Program to the last time-point of observation of 28 years, with the exception that there were no guidelines for HPV testing before 2005. All subsequent tests were registered in SymPathy.

2.3 Inclusion criteria

All women referred to the HPV outpatient clinic at UNN Tromsø in 1990-1992, having an inclusion HPV test during that time, were included in this retrospective cohort. The selected group of women all had a variety of symptoms from the lower urogenital tract, and had one or more cytology- and biopsy-specimens taken.

2.4 Exclusion criteria

After identifying all inclusion HPV-tests eligible for study participation, we excluded the few men present. In some cases, there was no match between the patient and the cervical cytology with the following HPV test. These patients were excluded. Using four years quarantine from baseline, we also excluded women having prevalent CIN2+ already at time of the inclusion HPV test.

2.5 Methods

All women that had a cervical specimen collected, either cytological or histological sample, were identified, and data was saved in a file with specimen as unit of registration. Within this file, each woman was given a pseudonymous number. HPV-testing was done by a two-step nonradioactive DNA hybridization method (ONCOR). In addition, a polymerase chain reaction (PCR) method using papilloma consensus primers was performed. HPV-types identified were 6, 11, 16, 18, 31 and 33 causing 78.4% of all cervical cancer (4). A positive result was defined as positive in one or both methods. All the HPV-results were written by

hand, and had to be linked with the current cytology for each person. In SymPathy, using the unique Norwegian 11-digit personal identification number, it was possible to find the exact cytology and add the HPV result. During the period of follow-up we detected all incidents of CIN and cervical cancer within our study population, comparing HPV status at baseline with the incidence of CIN3+.

2.6 Statistical methods

We used Chi-square test, Student t-test and survival-analysis to describe differences between the two groups. All analyses were done by SPSS, version 22.0, with $p<0.05$ as the level of significance.

2.7 Ethics

Internal quality assurance work is not qualified as research and therefore no ethical approval was needed (2011/2397/REK nord). All participants at the HPV-outpatient clinic at UNN in 1990-1992 were informed about the purpose of the HPV sample, and that their results would be used for research. In this report, all data regarding the women are fully anonymous. Lists containing names, dates of birth, the unique Norwegian 11-digit personal identification numbers and the HPV results, are stored safely, and only the main supervisor has access. This report will focus on the association between high-risk HPV, precancerous lesions and cervical cancer. It does not involve any intervention to the women, nor would it have any consequence for the individual.

3 Results

3.1 Inclusion, exclusion and cohort characteristics

A total number of 642 women had an inclusion cytology or histology and were enrolled in the study. In our analysis we defined HPV positive as women with positive test for one or more of the high-risk HPV types (16, 18, 31 and 33). The HPV negative group included low risk HPV-types (6 and 11). At baseline, 223 women (34.7%) tested HR-HPV positive (case-

group), and 419 women (65.0%) had a negative HPV test (control-group). We examined the distribution of HPV types at baseline. The most common HR-HPV type was HPV16, which accounted for 66.4% of HPV positive women, followed by HPV33 (14.8%), HPV31 (12.1%) and HPV18 (6.7%). HPV16 was found to be the most carcinogenic of the viruses (5, 28).

3.2 Cumulative incidence of CIN2+ and CIN3+

During 28 years of follow-up, CIN2+ was detected in 200 out of 642 women, which gives a cumulative incidence of 31.2%. Of these, 158 were detected within three years after the HPV test was taken. For 26 women, CIN2+ was detected four years or more after the test. Among the 200 women with CIN2+, 127 women (63.5%) were HR-HPV positive, while 73 women (36.5%) were HR-HPV negative at baseline. The risk of CIN2+ among the HPV positive and HPV negative was 57.0% (127/223) and 17.4% (73/419), respectively ($p<0.01$). Of the 223 HR-HPV positive women, 102 (45.7%) developed CIN3+, while 44 (10.5%) out of 419 HPV negative women developed CIN3+ ($p<0.01$). The overall cumulative incidence of CIN3+ was 22.7%.

3.3 Cervical cancer

Our analysis detected seven cases of cervical cancer. Of the seven women with cervical cancer, four women (57.1%) were HR-HPV positive at baseline, while three women (42.9%) were HR-HPV negative. All HR-HPV positive women who developed cervical cancer were HPV positive for HPV type 16. They were 27, 31, 34 and 66 years old at the time of the cancer diagnosis. They developed cervical cancer 3, 5, 5, and 22 years after the positive HPV test. The HR-HPV negative women were 30, 32 and 51 years old. All three had prevalent cervical cancer at baseline.

3.4 HPV and cellular changes by age

The prevalence of HPV infection decreased with age. In the youngest age-group, 16-24 years, 46.1% were HR-HPV positive, while for women aged 34-69 years, only 23% were HR-HPV

positive ($p<0.01$). Table 1 and 2 present the incidence of CIN2+ and CIN3+ in the age-groups 16-24 and 34-69, respectively. Our analysis shows that the age-group 25-33 years, has the largest proportion of women with CIN2+ and CIN3+ ($p<0.01$). In our study, probably due to the low prevalence of cancer, the occurrence of cervical cancer has no significant correlation to age ($p=0.46$).

3.5 Survival analyses for CIN3+

The survival analyses were nearly equal for the outcome of CIN2+ and CIN3+. This means that most of the women with CIN2+ also developed CIN3+. Because of that, we have focused on CIN3+ as the main outcome. As we can see from the Kaplan-Meier curve illustrated in Figure 2 there were significantly more women with positive HPV test that developed CIN3+, compared to women with negative HPV test. The incidence was higher the first five years after HPV test. This means that the majority of CIN3+ were prevalent at baseline or occurred during the first years after baseline.

Another interesting result was the incidence of CIN3+ comparing HPV-negative, HPV16/18 positive and HPV31/33 positive. As we already know, there was a significant difference between HPV negative and HPV positive patients. Women with HPV 31/33 had the highest incidence of CIN3+, but there were few new events later than three years after baseline. Later during the period of follow-up, the incidence of CIN3+ was quite similar between HPV 16/18 and HPV 31/33. This is illustrated in Figure 3.

Common to all our analyses, there was a high incidence of women with CIN3+ at baseline, meaning they had prevalent CIN3+ already when the baseline HPV test was taken. HPV status at baseline could tell us something about the future risk of CIN3+ when we have ruled out all women with prevalent CIN3+ at the time of the baseline HPV test. We therefore performed survival analysis with four years quarantine from baseline.

3.6 Four years quarantine survival analysis CIN3+

Using a filter with four years quarantine from baseline, our referral population becomes more equivalent to a general screening population. Figure 4 illustrates the incidence of CIN3+ with four years quarantine from baseline. The incidence of CIN3+ was higher among HPV positive than HPV negative women, but the overall incidence during follow-up was significantly lower. The difference between HPV positive and HPV negative women regarding incidence of CIN3+ was also lower.

We compared the incidence of CIN3+ between HPV-negative, HPV 16/18-positive and HPV 31/33-positive women using four years of quarantine from baseline. The results are illustrated in Figure 5. The incidence of CIN3+ was highest among women with HPV 16/18. Another interesting finding is that the incidence of CIN3+ was quite similar for HPV negative and HPV 31/33-positive women when using four years of quarantine. This finding suggests that the long-term risk of CIN3+ is considerably lower among women with HPV 31/33 compared to HPV 16/18, even though the short term risk of CIN3+ in our population was high.

3.7 Long-term risk of CIN3+ comparing all HPV types

Finally, we performed analyses on the incidence of CIN3+ for every single HPV type, illustrated in Figure 6. Women with HPV33 had the highest incidence of CIN3+, but there were few new events three years after baseline. HPV16 was second for incidence regarding CIN3+. Unlike HPV33, HPV16 had a steady increase in incidence of CIN3+ beyond the first three years after baseline. HPV31 was third on our list for incidence of CIN3+. Finally, HPV18 followed with a considerably lower incidence of CIN3+. Our analysis showed that the incidence of CIN3+ was similar for women with negative HPV test and women with positive low-risk HPV test, 6 and 11. This means that detection of low-risk HPV types does not predict CIN3+.

4 Discussion

4.1 Important findings compared to relevant literature

During 28 years of follow-up, CIN2+ was detected in 200 out of 642 women, an overall cumulative incidence of 31.2%. Among the 200 women with CIN2+, 127 women (63.5%) were HR-HPV positive, while 73 women (36.5%) were HR-HPV negative at baseline. The risk of CIN2+ among the HPV positive and HPV negative women was 57.0% (127/223) and 17.4% (73/419), respectively ($p<0.01$). Among the 223 HR-HPV positive women, 102 (45.7%) developed CIN3+, while 44 (10.5%) out of 419 HPV negative women developed CIN3+ ($p<0.01$). The overall cumulative incidence of CIN3+ was 22.7%. Castle et. al estimated CIR of CIN2+ and CIN3+ in an American screening population to be 2.64 and 1.36, respectively, after 18 years of follow-up (25). This indicates that our study population had a significantly higher risk of CIN2+ and CIN3+, which is expected because they constitute a referred population.

The proportion of HPV positive women decreases with age. In the youngest age-group, 16-24 years, 46.1% were HPV positive, while for women aged 34-69 years, only 23% were HPV positive ($p<0.01$). In comparison, within the general screening population, approximately 30% of women under the age of 30 have an ongoing HPV infection. The same applies to 6-7% of women aged 34-69 years (29). This finding suggests that women participating in our study had a slightly higher incidence of HPV-infections compared to the general screening population. Nevertheless, one must take into consideration that HPV testing is not routinely used in the primary screening of women under the age of 34 years.

Further, our analysis showed that the highest incidence of CIN2+ and CIN3+ was found within the group of women 25-33 years of age, and that most women with prevalent CIN2+ also developed CIN3+. This findings matches the natural evolution of a persistent HPV infection, suggesting that detection of CIN3 occurs 9.4 years after HPV infection occurs (8).

We found that most of CIN3+ does not progress to cervical cancer, presumably because of treatment or regression due to an immune response. A 2019 study by McCredie et al. compared the long-term risk of invasive cancer of the cervix in women whose CIN3 lesion was minimally disturbed with those who had adequate initial treatment followed by conventional management. They found that women with untreated CIN3 were at high risk of cervical cancer, whereas the risk in women treated conventionally throughout were very low. The cumulative incidence of cervical cancer at 30 years were 31.3% and 0.7%, respectively (30).

The incidence of CIN3+ was significantly higher in women who were HR-HPV positive, compared to those who were HR-HPV negative. The majority of CIN3+ was detected within three years after the HPV-test was taken, suggesting that these women already had prevalent CIN3+ at baseline. To predict the long-term risk of CIN3+ we had to rule out everyone with prevalent CIN3+ during and four years after baseline. Using four years quarantine from baseline, the difference in incidence of CIN3+ was considerably lower when comparing HPV positive and HPV negative women. This finding could be related to the fact that our study does not investigate the persistence of HPV infections. It is likely that women with a negative HPV test at baseline still acquires a HPV infection during 28 years of follow-up. Using four years of quarantine from baseline, HPV 16/18 had the highest incidence of CIN3+, while the incidence was quite similar among HPV negative and HPV31/33-positive women. A study by Kjaer et. al discovered that HPV16, HPV18, HPV31 and HPV33 infection, and especially HPV16 persistence, were associated with high absolute risks for progression to high-grade cervical lesions. They also found that two years persistence of HPV16 carries a 50% risk of CIN 3 (5).

When removing the quarantine, women with HPV33 had the highest incidence of CIN3+, but there were few new events later than three years after baseline. HPV16 was second for incidence regarding CIN3+. Third on our list for incidence of CIN3+ we found HPV31. Finally, HPV18 followed with a considerably lower incidence of CIN3. Our result is supported by Sjoeborg et al., which found that HPV16 and HPV33 appeared to have a higher oncogenic

potential than other HPV types (31). A 2012 study by Tjalma et al. found that the most common HPV types in women with high-grade CIN were HPV 16/33/31, and in invasive cervical cancer HPV 16/18/45, supporting that HPV33 carries a high risk of CIN3+ even though the risk of cancer is considerably lower than HPV18 (9).

Our analysis showed that the incidence of CIN3+ was similar for women with a negative HPV test and women with a positive low-risk HPV test (6 and 11). Our findings are supported by Thomsen et al. who found that detection of low-risk HPV does not predict CIN3+. They suggested that cervical cancer screening should not include testing for low-risk HPV types (32). Ronco et al. discovered that a negative high-risk HPV test provides greater long-term reassurance against CIN3+ than normal cytology (16).

4.2 Strengths of the study

This study is a retrospective registry-based cohort with a prospective design and 28 years of follow-up. There are few other studies with this long time of follow-up, probably because HPV testing was not widely used in the 90s.

We could assume that the prevalence of CIN3+ is higher in our study population compared to an average screening population, and can thereby qualify as a referred population. The positive predictive value (PPV), the proportion of true positive test results, depends entirely on the disease prevalence within the group that is tested. In a screening population the disease prevalence is expected to be low, and the PPV will be low as well. In a referral population the disease prevalence is expected to be higher, and PPV will be high as well. Using four years quarantine from baseline our “referral population” is cleansed for CIN2+, while women with a HPV-infection and risk of CIN2+ in the future will be discovered later during follow-up.

Of all women with inclusion HPV-test, there were almost twice as many women with a negative HR-HPV test compared to women with a positive HR-HPV test (419 and 223 women, respectively). This is a relatively large population.

4.3 Limitations and weaknesses of the study

As mentioned previous our referred population is a limitation of the study, making the results less representative for a general screening population. Unfortunately our study has no data on persistence of HPV infections because HPV testing was not a part of the routine follow-up through the NCCSP and because the HPV project at UNN was terminated earlier than planned.

4.4 Implications of findings

Our findings do not involve new research, but support already established knowledge. New and future studies have the opportunity to look at HPV infections and the role of persistence. Improved assessment of HPV persistence by HPV testing in primary screening, would make future studies easier to perform as registry-based. Our study has only one HPV test at baseline, and we can only assume that women with detected CIN2+ had persistence of HPV infections throughout many years.

5 Conclusion

HPV-status at baseline is predictive for women´s subsequent risk of developing high-grade CIN. Women with a positive HPV-test in 1990-1992 had a significantly higher risk of CIN3+ during 28 years of follow-up compared to HR-HPV negative women. The cumulative incidence of CIN3+ within the two groups were quite similar to that of CIN2+, suggesting that most women with prevalent CIN2+ also developed CIN3+.

The majority of CIN3+ were prevalent at baseline or occurred within the first years afterwards. Women aged 25-33 years had the highest incidence of CIN2+ and CIN3+. Women with HPV 31/33 had the highest incidence of CIN3+ the first few years after baseline. Using four years of quarantine from baseline, the incidence of CIN3+ was highest among women with HPV16/18, and similar between HPV 31/33 and HR-HPV negative, suggesting that the long-term risk of CIN3+ is considerably lower among women with HPV 31/33 compared to HPV 16/18, even though the short-term risk of CIN3+ in our population was high.

Women with HPV33 had the highest risk of developing CIN3+, followed by HPV16, HPV31 and finally HPV18. Regardless, there were few new events of CIN3+ among HPV33-positive beyond three years after baseline, supporting our statement above. The incidence of CIN3+ was similar for women with negative HPV-test and women with positive low-risk HPV-test, including HPV6 and HPV11, suggesting that detection of low-risk HPV-types does not predict CIN3+ and therefore should be omitted from primary screening for cervical cancer.

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7 Tables and figures

Age (years)	Histological confirmed CIN2+		
	No	Yes	Total
16-24	144 (74.6%)	49 (25.4%)	193
25-33	103 (53.6%)	89 (46.4%)	192
34-69	188 (75.2%)	62 (24.8%)	250
>70	7 (100%)	0 (0,0%)	7
Total	442 (68.8%)	200 (31.2%)	642

Table 1: Incidence of CIN2+ in the different age-groups during 28 years of follow up.

Age (years)	Histological confirmed CIN3+		
	No	Yes	Total
16-24	158 (81.9%)	35 (18.1%)	193
25-33	123 (64.1%)	69 (35.9%)	192
34-69	208 (83.2%)	42 (16.8%)	250
>70	7 (100%)	0 (0.0%)	7
Total	496 (77.3%)	146 (22.7%)	642

Table 2: Incidence of CIN3+ in the different age-groups during 28 years of follow up.

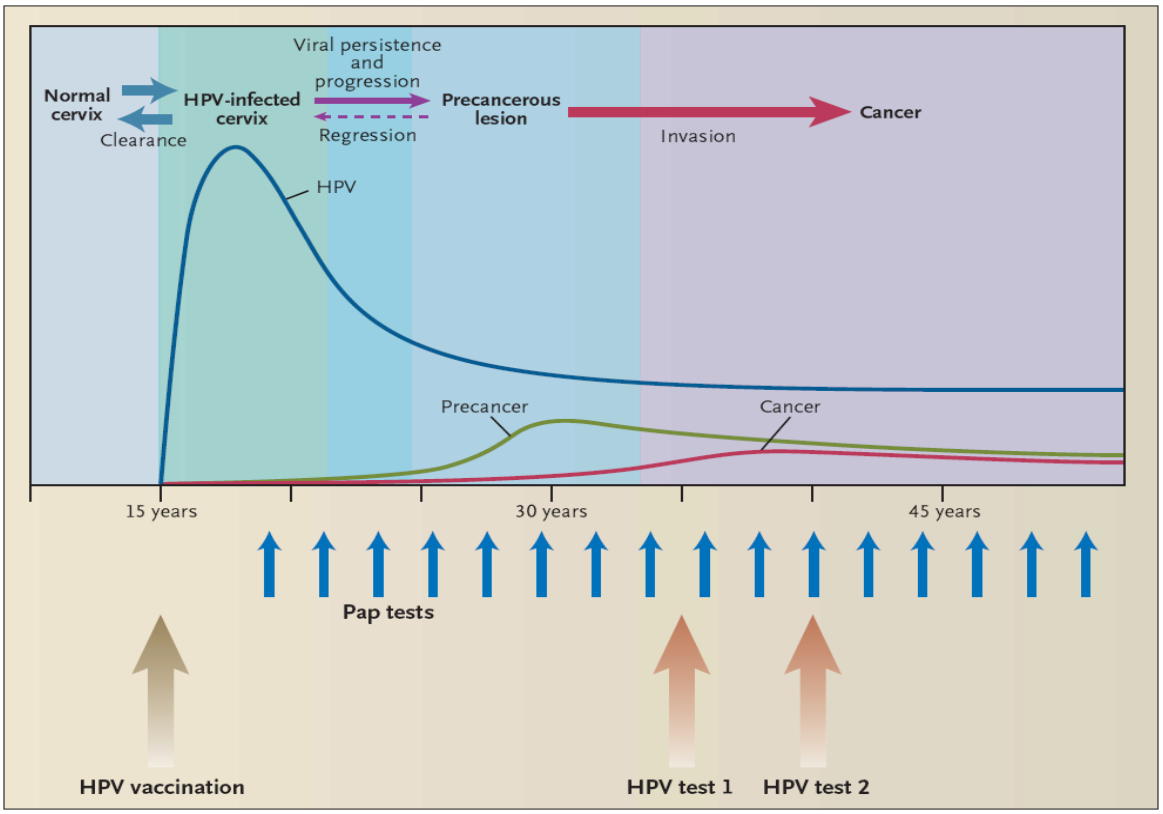

Figure 1: The Natural History of HPV infection and Cervical Cancer (33).

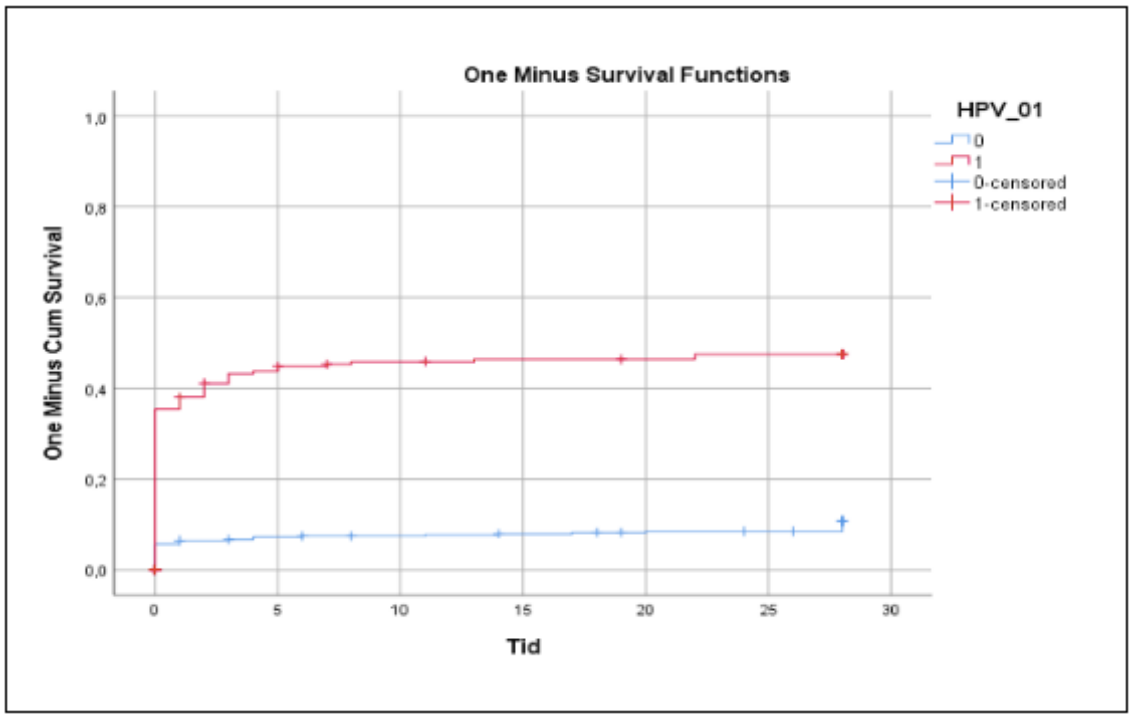

Figure 2: Cumulative incidence of CIN3+ by years of follow-up comparing the exposed cohort of women with a positive HR-HPV test at baseline (red) and the control cohort of women with a negative HR-HPV-test (blue).

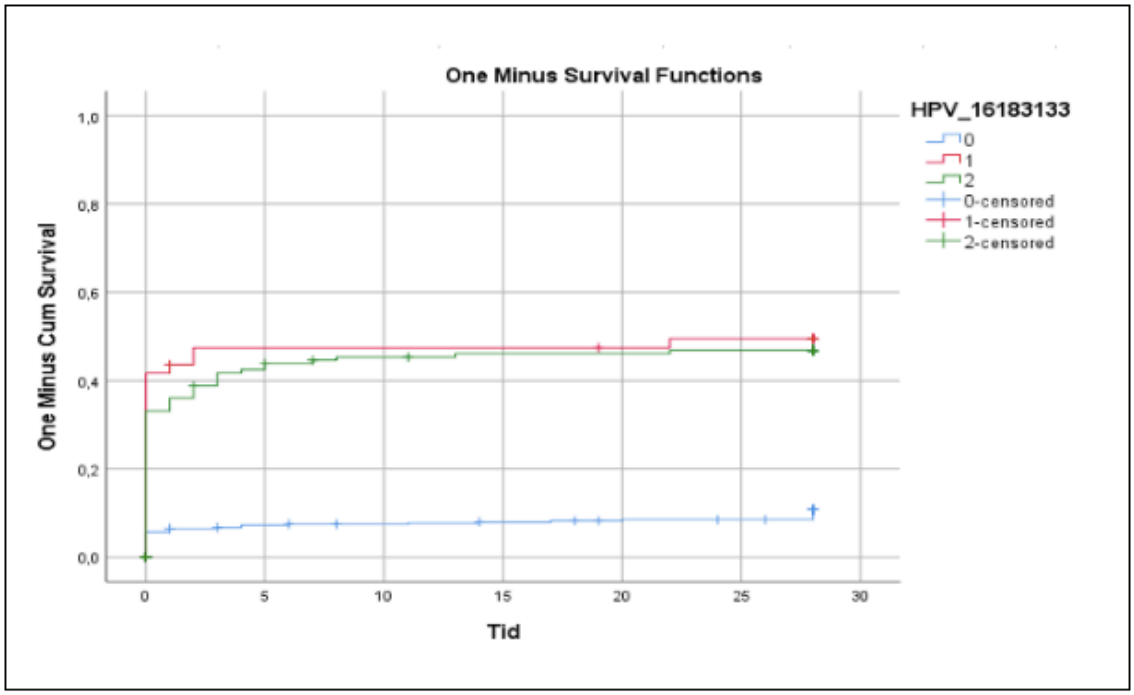

Figure 3: Cumulative incidence of CIN3+ by years of follow-up comparing the exposed cohort of women with HPV31/33 (red), HPV16/18 (green) and the control cohort (blue).

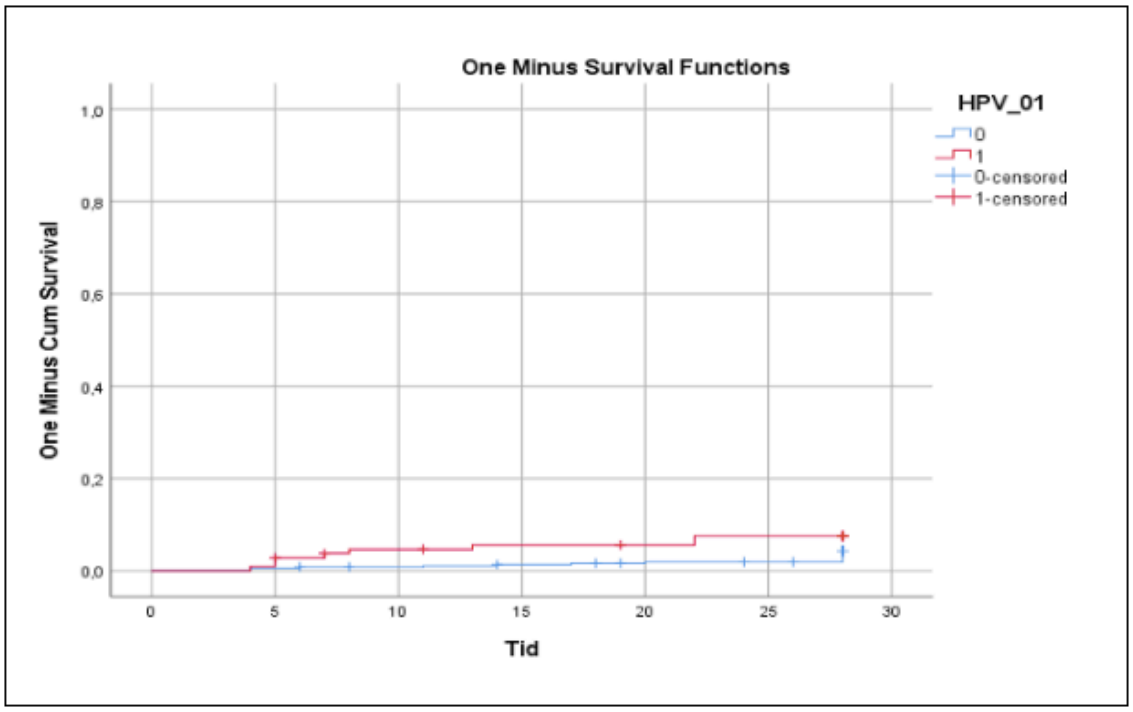

Figure 4: Cumulative incidence of CIN3+ by years of follow-up using four years quarantine from baseline, comparing the exposed cohort of women with a positive HR-HPV test (red) and the control cohort of women with a negative HR-HPV test (blue).

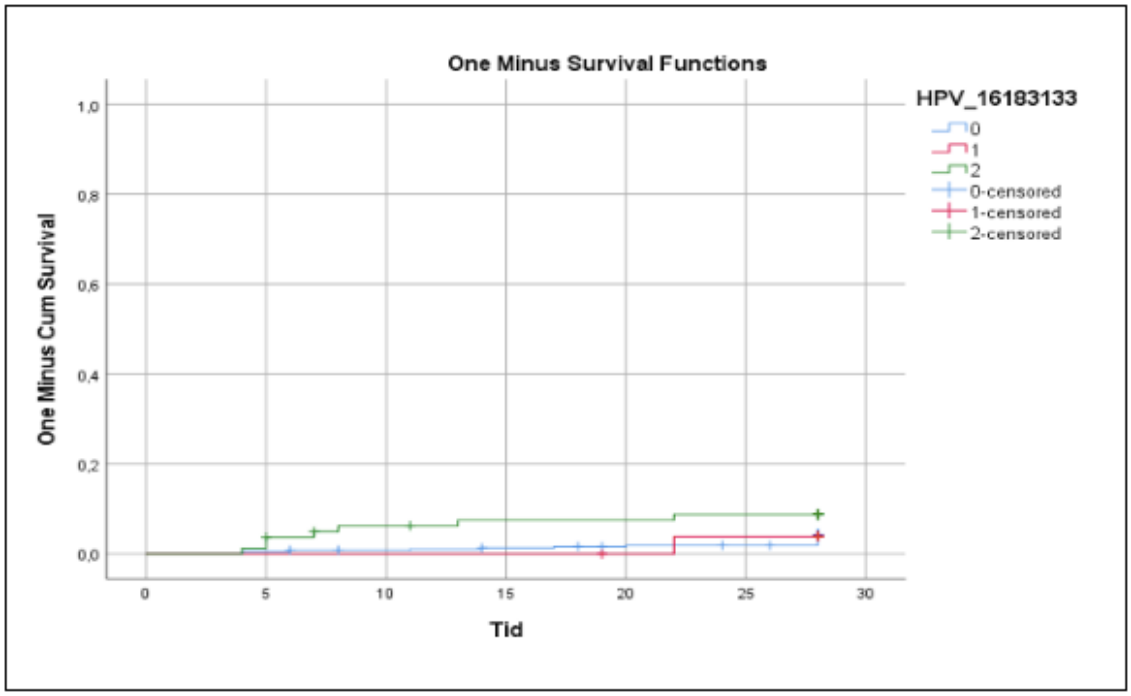

Figure 5: Cumulative incidence of CIN3+ by years of follow-up using four years quarantine from baseline. Comparing the exposed cohort of women with HPV16/18 (green), HPV 31/33 (red) and the control group of women with a negative HR-HPV test (blue).

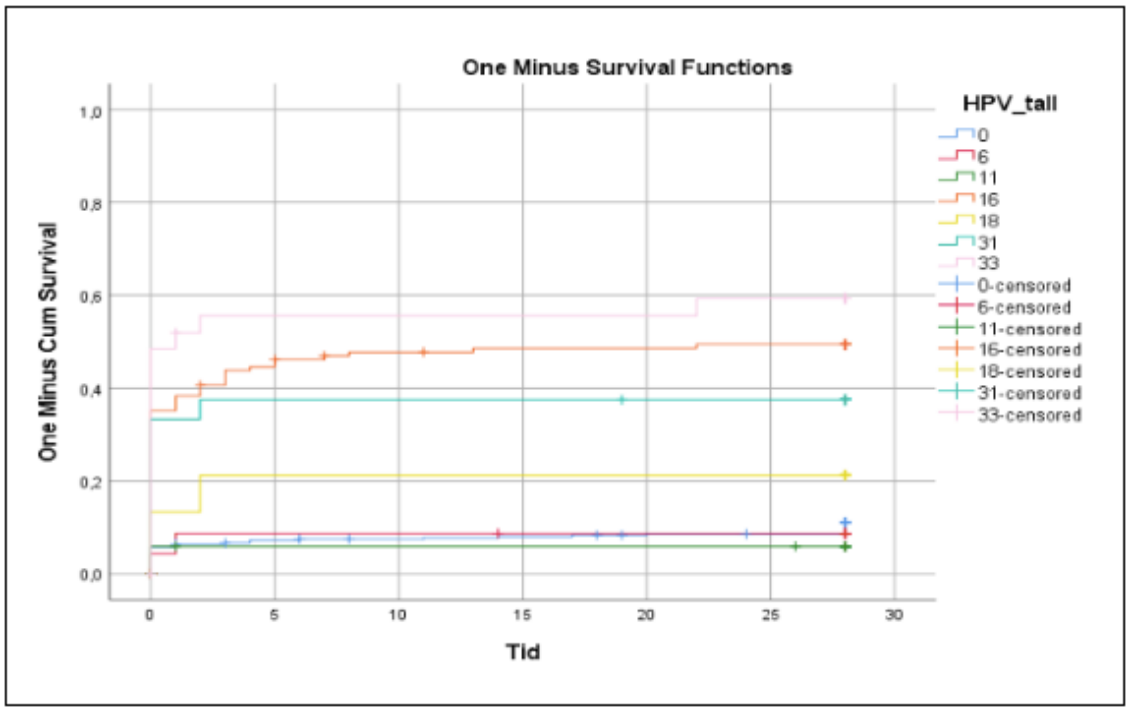

Figure 6: Cumulative incidence of CIN3+ by years of follow-up comparing every HPV type studied. HPV33 (pink), HPV16 (orange), HPV31 (light green), HPV18 (yellow), HPV6 (red), HPV11 (dark green) and negative HPV test (blue).

8 Summaries and evaluation of literature

Reference:
Castle PE, Glass AG, Rush BB, et al. Clinical human papillomavirus detection forecasts cervical cancer risk in women over 18 years of follow-up. J Clin Oncol 2012; 30: 3044-50.

Study design: Cohort

Grade – quality: Medium

Study objective	Material and methods	Results	Discussion/comments/checklist:
To describe the long term (> 10 years) benefits of clinical human papillomavirus (HPV) DNA testing for cervical precancer and cancer risk prediction. **Conclusion** ***HPV testing to rule out cervical disease followed by Pap testing and possible combined with the detection of HPV16 and HPV18 among HPV positives to identify those at immediate risk of CIN3+ would be an efficient algorithm for cervical cancer screening, especially in women age 30 years or older.*** **Country** United States of America **Year of data collection** From April 1, 1989, to November 2, 1990.	**Population:** 23 702 non-pregnant women, age 16 years and older, receiving apparently routine cytologic screening in a prepaid health plan at Kaiser Permanente in Portland, Oregon from April 1, 1989, to November 2, 1990. A total of 22 595 women (86.4%) agreed to participate. A final analytic cohort of 19 512 women was defined after exclusions. Of those in the analytic cohort, 4098 women (21.0%) had at least one screen 15 years or later after the cohort was initiated. **Cohorts:** four cohorts by baseline test results; HR-HPV positive, HR-HPV negative, ASC-US+ Pap and normal Pap. Throughout the analysis, they evaluated baseline test results individually and as paired HR-HPV test and Pap results (HR-HPV positive/ASC-UC+, HR-HPV positive/normal, HR-HPV negative/ASC-US+, HR-HPV negative/normal). **Main outcome:** occurrence of CIN3+ over the 18 years of follow up. **Important confounding factors:** Converting Pap terminology from a previous classification to the 2001 Bethesda System. **Statistical methods:** Using the Kaplan-Meier method, they calculated cumulative incidence rates (CIRs) with 95% confidence intervals for each interval up to the end of the observation time.	**Main findings:** A baseline negative HPV test provided greater reassurance against CIN3+ over the 18-years of follow-up than a normal Pap (CIR, 0.90% v 1.27%). Although both baseline Pap and HPV tests predicted who would develop CIN3+ within the first 2 years of follow-up, only HPV-testing predicted who would develop CIN3+ 10 to 18 years later (P=0.004). **Rate/proportion/ratio/rate difference** There were 396 patient cases of CIN2+ and 199 of CIN3+ diagnosed over the 18 years of the study. More patient cases of CIN2+ (215 v 136; P<0.001) and CIN3+ (112 v 65; P<0.001) occurred after baseline HR-HPV-positive result versus positive Pap. Among HR-HPV–positive women, approximately half of those with CIN2+ and CIN3+ had concurrent negative baseline Pap. **Relative risk (RR):** 1.27/0.90=1.4. There is a 1.4 times increased risk of developing CIN3+ followed by a normal baseline Pap-smear compared to a baseline negative HPV-test. **Absolute Risk Reduction (ARR):** 1.27%-0.90%=0.37%. **Confidence intervals (CI):** Narrow confidence intervals suggesting that the estimates are reliable. **Dose-response:** Cervical cancer risk increased with more severe Pap interpretations and higher risk HPV genotypes. Women who tested HPV16 positive had a similar or higher 18-year CIR than women with any Pap interpretation other than HSIL. **Other findings:** After negative HPV and Pap tests in women age 30 years and older, the 3-year risk of CIN2+ and CIN3+ were 0.23% and 0.08%, respectively. If the screening intervals were extended to 5 years, the risk were 0.36% and 0.16%, respectively.	• **Was the purpose clearly stated?** Yes • **Are the cohorts recruited from the same population?** Yes • **Selection bias?** No. Only 4.7% of women approached during 1989/1990 refused. • **Were the exposed individuals representative of a defined population?** Yes • **Were exposure and outcome measured equally and reliable (validated) in the two groups?** Yes • **Was the one who evaluated the results (endpoints) blind to group affiliation?** No • **Was the study prospective?** Yes • **Were enough persons in the cohort followed up?** Yes, 82.3% were in the analytic cohort, whereas only 21.0% underwent screening 15 years or later after enrolment. • **Were apostasy analyses performed?** No • **Was the time of follow-up long enough to detect positive and/or negative outcomes?** Yes, until 214 months (approximately 18 years). • **Are important confounding factors in design/ implementation/analyses taken into account?** Yes • **Do you believe in the results?** Yes • **Can the results be transmitted to the general population?** Yes • **Other literature that strengthens and weakens the results?** Yes, one of them is Dillner et. al who also found that the CIR of CIN3+ after six years was considerably lower among women negative for HPV at baseline than among women with negative results on cytology. Ronco et. al suggested that HPV-based screening provides 60-70% greater protection against invasive cervical carcinomas compared with cytology, supporting the results in this study. • **Implications of the findings?** Provides additional support for the use of HPV testing in routine screening in women age 30 years or older. **Described strengths:** large study population. **Described weaknesses:** Old guidelines on indications for colposcopy. Use of old Pap terminology (their ASC-US does not perfectly represent current-day ASC-US). Also HPV testing was slightly less sensitive for HPV and related lesions. For example, only 38 (74.5%) of 51 baseline HSIL cytology results tested HC2 positive, rather than the expected 90% to 95%.

Reference: Dillner J, Rebolj M, Birembaut P, et al. Long term predictive values of cytology and human papillomavirus testing in cervical cancer screening: joint European cohort study. Bmj 2008; 337: a1754.

Study design: Cohort (multinational)

Grade – quality: Medium

Study objective

To obtain large scale and generalisable data on the long term predictive value of cytology and human papillomavirus (HPV) testing for development of cervical intraepithelial neoplasia grade 3 or cancer (CIN3+).

Conclusion

A consistently low six year cumulative incidence rate of CIN3+ among women negative for HPV suggests that cervical screening strategies in which women are screened for HPV every six years are safe and effective.

Country

Primary data from seven HPV screening studies in six EU countries (Denmark, Germany; Hannover and Tübingen, United Kingdom, France, Sweden, Spain) were used in this multinational cohort study.

Year of data collection

Denmark: 1993-1995.
Germany: 1999-2000
United Kingdom: 1994-1997
France: 1997-2002
Sweden: 1997-2000
Spain: 1997-2001

Material and methods

Population: 24 295 women attending cervical screening enrolled into HPV screening trials in one of six European countries, who had at least one cervical cytology or histopathology examination during follow-up.

Cohorts: Original baseline groups:
Cytology-/HPV- (No at baseline: 21 060)
Cytology-/HPV+ (No at baseline: 1962)
Cytology+/HPV- (No at baseline: 436)
Cytology+/HPV+ (No at baseline: 837)

Main outcome:
Long term cumulative incidence (CIR) of CIN3+.

Important confounding factors: the cumulative incidence rate of CIN3+ at 60 months, among women with positive cytology and HPV test, was clearly different between the countries.

Statistical methods: estimation of the specific cumulative incidence rate of CIN3+ by original baseline group, as mentioned previous, for each country, with 95% confidence intervals, using the Kaplan-Meier product limit estimator for log(hazard). They used comparative analysis of systematically drawn subsamples of the joint cohort (bootstrap analysis) to determine whether lack of homogeneity between the different studies in the joint cohort. Thirdly they calculated the test performance indices for cytology alone, HPV test alone, and cytology and HPV test combined, using 2x2 tables based on the cumulative incidence rate at 72 months for the different baseline test combinations.

Results

Main findings: The cumulative incidence rate of CIN3+ after six years was considerably lower among women negative for HPV at baseline (0.27%, 95% CI 0.12% to 0.45%) than among women with negative results on cytology (0.97%, 0.53% to 1.34%). The CIR among women with negative cytology results who were positive for HPV increased continuously over time, reaching 10% at six years, whereas the rate among women with positive cytology results who were negative for HPV remained below 3%.

Between exposed/unexposed: The positive predictive value for future CIN3+ was highest among women with cytology+/HPV+ at baseline, CIR 34% (95% CI 28.6% to 45.4%). Women with cytology-/HPV+ had a continuously increasing CIR of CIN3+, eventually reaching 10% (6.2% to 15.1%) after six years. Women with cytology+/HPV- had a CIR for CIN3+ of 2.7% (0.6% to 6.1%). Women with both normal cytology and negative HPV test (cytology-/HPV-) had a low risk of future CIN3+ (0.28%, 0.10% to 0.47%).

Relative Risk (RR): 0.0097/0.0027=**3.6.**
Absolute Risk Reduction (ARR): 0.97%-0.27%=0.70%.

Confidence intervals:
Relatively narrow and therefore little uncertainty

Dose-response: the cumulative incidence of CIN3+ among women positive for HPV was lower than for women with abnormal cytology but increased continuously and gradually during months.

Additionally findings: both cytology and HPV test had higher specificity for women above 35 years but did not improve any further among women >49

Discussion/comments/checklist:

Checklist:

- **Was the purpose clearly stated?** Yes
- **Are the cohorts recruited from the same population?** Yes
- **Selection bias?** No
- **Were the exposed individuals representative of a defined population?** Yes of women attending cervical screening.
- **Were exposure and outcome measured equally and reliable (validated) in the two groups?** Yes.
- **Was the one who evaluated the results (endpoints) blind to group affiliation?** Unkown.
- **Was the study prospective?** Yes.
- **Were enough persons in the cohort followed up?** Yes
- **Were apostasy analyses performed?** No
- **Was the time of follow-up long enough to detect positive and/or negative outcomes?** Yes, but could have been longer.
- **Are important confounding factors in design/ implementation/analyses taken into account?** Yes
- **Do you believe in the results?** Yes
- **Can the results be transmitted to the general population?** Yes, it is representative for all women attending cervical screening.
- **Other literature that strengthens and weakens the results?**
- **Implications of the findings:** suggests that screening intervals could safely be lengthened to six years among women with negative result on an HPV test.

Described strengths: that several studies in different settings in different countries and with different infrastructure and intensity of follow-up gave largely similar results implies that the data are generalisable to various settings. Also that they studied the actual cytological tests used in the different countries implies that the data are generalisable.

Described weaknesses: verification bias might overestimate the performance of screening tests when only women with a positive screening test result are referred for colposcopy.

Reference:
Kjaer SK, Frederiksen K, Munk C, et al. Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. J Natl Cancer Inst 2010; 102: 1478-88.

Study design: Cohort

Grade – quality: Medium

Study objective	Material and methods	Results	Discussion/comment/checklist
Long-term risk of high-grade CIN after one-time detection of high-risk HPV DNA and after persistent infection with individual high-risk HPV types. **Conclusion** ***HPV16, HPV18, HPV31 and HPV33 infection and especially HPV16 persistence were associated with high absolute risk for progression to high-grade cervical lesions.*** **Country** Denmark **Year of data collection** Between May 15, 1991, and January 31, 1993. From October 1, 1993, to January 31, 1995, the study participants were re-invited.	**Population:** 11088 women who were 20-29 years of age at enrolment, selected at random from the general female population of Copenhagen, Denmark. 8656 women (76%) participated in both gynaecological examinations. 381 women, who participated in the second examination only through a telephone interview, were excluded, as well as 193 women with an abnormal smear at baseline, 47 women who had had an abnormal smear within 1 year before baseline, and 356 women for whom no cervical swab was available at the baseline visit. Finally they excluded 197 women who did not have any gynaecological examination after baseline, leaving 7482 women who were included in the analysis. **Cohorts:** Of the 7482 women with a normal Pap smear at baseline who were included in the study, 1281 (17.1%) were positive for high-risk HPV DNA (exposed cohort). 6201 (86.9%) women were negative for high-risk HPV DNA (control cohort). **Main outcome:** Cervical Intraepithelial Neoplasia (CIN) grade 3 or worse. **Important confounding factors:** The histological diagnosis were translated into CIN nomenclature as follows: moderate dysplasia as CIN2 and severe dysplasia and carcinoma in situ as CIN3. **Statistical methods:** The absolute risk of developing cervical lesions was estimated as a function of time by interval-censured observations. In the analysis related to persistence of individual HPV types, pointwise 95% CIs were calculated.	**Main finding:** For women with normal cytological findings who were concurrently HPV16 DNA positive at the second examination, the estimated probability of developing CIN grade 3 (CIN3) or worse within 12 years of follow-up was 26.7% (95% CI= 21.1% to 31.8%). The corresponding risk among those infected with HPV18 was 19.1% (CI=10.4% to 27.3%), with HPV31 was 14.3% (CI = 9.1% to 19.4%), and with HPV33 was 14.9% (CI = 7.9% to 21.1%). **Between exposes/unexposed:** The absolute risk of CIN3 or worse following a negative HPV-test was 3.0% (CI = 2.5% to 3.5%). One positive test and persistence of high-risk HPV types other than HPV16, HPV18, HPV31, and HPV33 were associated with low absolute risks of CIN3 or worse that lasted for years. HPV negative women stayed at very low risk of CIN3. **How strong is the association (RR)?** 0.267/0.030=8.9. A strong association. **What is the absolute risk reduction (ARR)?** 0.267-0.030=0.237 (23.7%). **Confidence Intervals (CI)** Significant, relatively narrow. **Dose-response:** the role of persistence of HPV infection is significant, the longer persistence of infection the higher the risk of developing CIN3. Also the HPV type prevalent is important to predict the risk of future high-grade CIN.	• **Was the purpose clearly stated?** Yes • **Are the cohorts recruited from the same population?** Yes • **Selection bias?** Volunteer participants in a study can differ from those who do not want to participate. Normal cytology was a prerequisite for participation, and that minimizes the risk of selection bias in this study. • **Were the exposed individuals representative of a defined population?** Yes, for the general female population in Copenhagen, Denmark, who were 20-29 years and had a normal cytology at enrolment. • **Were exposure and outcome measured equally and reliable (validated) in the two groups?** Yes • **Was the one who evaluated the results (endpoints) blind to group affiliation?** • **Was the study prospective?** Yes • **Were enough persons in the cohort followed up?** Yes. • **Were apostasy analysis performed?** No, but the cohort was followed up passively through the Pathology Data Bank, personal identification numbers made it possible to conduct follow-up studies with virtually no loss to follow up. • **Was the time of follow-up long enough to detect positive and/or negative outcomes?** A study from 2012 by Depuydt et. al found that detection of CIN3 occurs 9.4 years after HPV-infection, so yes, 13 years of follow up should often be long enough to detect positive and negative outcomes. • **Are important confounding factors in design/implementation/analyses taken into account?** Yes. • **Do you believe in the results?** Yes, good causality. • **Can the results be transmitted to the general population?** Yes • **Literature that strengthens or weakens the results?** Yes, mostly strengthens. • **Implications of findings?** These findings may be useful in the development of more specific cervical cancer screening methods, identify issues that need to be resolved to obtain the greatest clinical value from HPV testing, and/or be of value in the development of new generations of prophylactic HPV vaccines and suggest that cervical cancer screening intervals for HPV-negative women could be prolonged. **Strengths:** identifying the role of persistence, appropriate time of follow up. **Weaknesses:** Rates of progression of some HPV types after persistence may have been overestimated because some of them might have been re-infections with the same HPV type.

Reference:
McCredie MR, Sharples KJ, Paul C, et al. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. Lancet Oncol 2008; 9: 425-34.

Study design: Cohort

Grade – quality	Medium

Study objective	Materials and methods	Results	Discussion/comments/checklist:
Compare the long-term risk of invasive cancer of the cervix in women with CIN3 whose lesions was minimally disturbed with those who had adequate initial treatment. **Conclusion** ***Women with untreated CIN3 are at high risk of cervical cancer, whereas the risk is very low in women treated conventionally throughout.*** **Country** New Zealand **Year of data collection** Between February, 2001, and December, 2004.	**Population:** 1229 women with diagnosed CIN3 at the National Women´s Hospital, Auckland, between Jan 1, 1955 and Dec 31, 1976, whose treatment was reviewed by the judicial inquiry in 1987-88 were included. Of these 48 records (4%) could not be located and 47 women (4%) did not meet the inclusion criteria. At histopathological review, a further 71 (6% of 1134) women were excluded because the review diagnosis was not CIN3. The study identified outcomes in the remaining 1063 (86% of 1229) women diagnosed with CIN3 at the hospital in 1955-76. **Cohorts:** An exposed cohort of women whose CIN3 lesions was minimally disturbed compared with those who had adequate initial treatment for CIN3 in the same period. **Main outcome:** Cumulative incidence of invasive cancer of the cervix or vaginal vault. **Important confounding factors:** New cytological and histological classification systems since the time of diagnosis. Also the study was made possible by the existence of an unethical clinical study, in which treatment was often withheld or delayed. **Statistical methods:** Kaplan-Meier survival methods were used to estimate cumulative proportion of (first) cancer of the cervix or vaginal vault. 95% CI were calculated by use of the log (-log of the survival function). Groups were compared by the log-rank test, and hazard ratio (HRs), designated here as relative risks (RRrs) with their 95% CI, estimated by use of Cox regression. A significance level of 0.05 was used throughout.	**Main finding:** In 143 women managed only by punch or wedge biopsy, cumulative incidence of invasive cancer of the cervix or vaginal vault was 31.3% (CI 22.7-42.3) at 30 years. In comparison, cancer risk at 30 years was only 0.7% (CI 0.3-1.9) in 593 women whose initial treatment was deemed adequate or probably adequate. **Rate/proportion/ratio/rate difference** 31 out of 143 (21.6%) women with minimum disturbance of the CIN3 lesion developed cancer of the cervix or vaginal vault. To comparison, 5 out of 593 (0.84%) women with adequate or probably adequate initial treatment developed cancer. **How strong is the association (RR)?** 31.3%/0.7%=44.7%. The relative risk of invasive cancer of the cervix or vaginal vault were 44.7 for women whose maximum initial procedure was punch or wedge biopsy (p=0.002). **What is the absolute risk reduction (ARR)?** 0.313-0.007=0.306 (30.6%) **Confidence interval (CI)** 95% CI was relatively wide for cumulative incidence of invasive cancer among women with inadequate treatment, whereas the 95% CI was narrow for cumulative incidence of invasive cancer among women whose initial treatment was deemed adequate or probably adequate. None including 1.0, and therefore the results were significant.	**Checklist:** • **Was the purpose clearly stated?** Yes • **Are the cohorts recruited from the same population?** Yes • **Selection bias?** No • **Were the exposed individuals representative of a defined population?** Yes, they are representative of an untreated population of women with CIN3. • **Were exposure and outcome measured equally and reliable (validated) in the two groups?** Yes • **Was the one who evaluated the results (endpoints) blind to group affiliation?** No • **Was the study prospective?** Yes • **Were enough persons in the cohort followed up?** Yes, 86%. • **Were apostasy analyses performed?** No • **Was the time of follow-up long enough to detect positive and/or negative outcomes?** Yes, a study by Tjalma et. al reveals that development from CIN3 to cervical cancer takes 10-20 years, depending on genotype. Follow-up continued until death or Dec 31, 2000, whichever came first. • **Are important confounding factors in design/ implementation/analyses taken into account?** Yes • **Do you believe in the results?** Yes • **Can the results be transmitted to the general population?** Partial, but the findings might be more applicable to a previously unscreened cohort of women. • **Other literature that strengthens and weakens the results?** • **Implications of the findings?** Direct estimates of the rate of progression from CIN3 to invasive cancer. Supports conventionally treatment of CIN3. **Described strengths:** low risk of false positive smears 6-24 months after any procedure. Classification of adequacy of treatment. **Described weaknesses:** As records of clinical follow-up became less complete during the late 1980s and the 1990s, some women might have had unrecorded treatment during later follow-up.

Reference: Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014; 383: 524-32.

Study design: RCT

Grade – quality: Medium

Study objective	Materials and methods	Results	Discussion/comments/checklist
Relative efficacy of HPV-based versus cytology-based screening for prevention of invasive cervical cancer in women who undergo regular screening. **Conclution** ***Women with negative HPV test have 60-70% lower risk of cervical cancer than women with normal cytology, after 6.5 years of follow-up.*** **Country** Italy, based on data from Italy, Sweden, England and Netherland **Year of data collection** Italy: 2002-2004 Sweden: 1997-2000 England: 2001-2003 Netherland: 1999-2002 This is a follow-up study of the four randomised trials.	**Recruitment of participants:** This study investigates the outcomes in four European RCT´s. The women recruited to all four trials had not had a hysterectomy and were attending for routine screening within organised population-based programmes. **Exclusion criteria:** Hysterectomy inn all studies. Women were excluded from NTCC if they were pregnant or treated for CIN in the previous 5 years, from POBASCAM if they had CIN2+ or abnormal cytology detected in the previous 2 years. No exclusion criteria were used at recruitment in Swedescreen and ARTISTIC. **Data-material:** Overall, 176 464 women were enrolled. After enrolment, women were randomly assigned to either HPV-based or cytology-based screening in a 1:1 ratio, except in England (3:1 ratio). **Outcome validation:** Potential cases of invasive cervical cancer arising during follow-up were identified in several ways, depending on the patient´s location. Cervical carcinomas were classified by morphological features – if possible, as squamous-cell carcinoma or adenocarcinoma, and by FIGO-stage. **Exposure variables:** None **Statistical methods:** cumulative incidence of invasive cervical cancer using the Kaplan-Meier method.	**Main finding:** Detection of invasive cervical carcinoma was similar between screening methods during the first 2.5 years of follow-up (0.79, 0.46-1.36) but was significantly lower in the experimental arm (HPV-based screening) thereafter (0.45, 0.15-0.60). This is a matter of relative detection of cancer in the HPV-arm versus cytology-arm, in which the detection of cancer was lowest in the HPV-arm. The cumulative incidence of invasive cervical carcinoma in women with negative entry tests was 4·6 per 10^5 (1·1–12·1) and 8·7 per 10^5 (3·3–18·6) at 3·5 and 5·5 years, respectively, in the experimental arm, and 15·4 per 10^5 (7·9–27·0) and 36·0 per 10^5 (23·2–53·5), respectively, in the control arm. **Cumulative detection rate:** similar in both arms up to about two years from enrolment, but diverged thereafter, reaching 46.7 per 10^5 (95% CI 32.1-65.5) in the experimental arm and 93.6 per 10^5 (70.5-121.8) in the control arm 8 years after enrolment. **Relative risk:** (44/94639)/(63/81825)=0.60. The rate ratio for invasive cervical carcinoma among all women from recruitment to end of follow-up was 0.60 (95% CI 0·40–0·89). Risk reduction= 1-(0.000464/0.000769)=0.40, suggesting that HPV tests prevents 40% more cases of cervical carcinoma than cytology. **Additionally findings:** In the first round of screening, 0.79 times as many cases of cervical cancer were found in the HPV arm than in the cytology arm. In fact, HPV testing may appear to have lower sensitivity than cytology in finding women with cancer, but the differences were not significant (0.79, 0.46–1.36). Beyond the first 2.5 years the confidence intervals does not include 1.0 and the findings are therefore significant.	• **Is the purpose clearly stated?** Yes. **Was the groups alike in the beginning?** Yes • **Who is included/excluded?** Women from four European countries who were attending for routine screening within organised population-based programmes, all randomly assigned. • **Randomization procedure?** In all four countries, except in one of the Italian centres, central computers did the randomisation of women to either HPV-based or cytology-based screening. • **Were participants/study staff blinded regarding group affiliation?** Yes the study staff, but not the participants. • **Were the groups treated equally beyond the "intervention"?** The studies used different screening protocols, but the HPV- and cytology arm were treated equally. • **Primary endpoint – Validated?** Yes, as described earlier, the primary endpoint was invasive carcinoma of the cervix and outcome validation was done in all four countries. • **Were participants accounted for in the end of the study?** Yes, few lost to follow up. • **What is the results? Plausible explanations?** Results shows that women with a negative HPV test have 60-70% lower risk of cervical cancer than women with normal cytology after 6.5 years of follow-up. The first 2.5 years of follow-up there is not a significantly difference between between screening methods. • **Can the results be converted into practice?** We know that HPV screening finds significantly more cases of CIN2/3 in the first screening round, so that there are fewer cases of cancer in the next screening round. This study showed the opposite, cytology found more cases of baseline cancer than HPV test. If the HPV test finds fewer cases of cervical cancer in the first screening round, we can not necessarily trust that fewer findings of cancer in the second round of screening are due to higher sensitivity because it may be the same women with cancer who are lost twice. • **Were all outcome measures considered?** Yes • **Are benefits worth the disadvantages/costs?** If the HPV test prevents 40% more cases of cancer than cytology, it is unknown what happens when we increase the screening interval by 67% from three years to five years. If a woman has a positive HPV test, it is not certain that she will receive treatment. For a woman with cancer, it does not help that the HPV test was positive five years ago if her cancer was not prevented. All cases of cancer after 2.5 years in the HPV-arm among HPV-positive must be considered a failure in the follow-up of women with a positive HPV test. **Strengths:** golden standard for this study objective, few lost to follow-up and no selection-bias. **Weaknesses:** Focusing on women with a negative screening test, comparing HPV test and cytology using 6.5 years screening intervals (which is unusual).