Untreated cervical intraepithelial neoplasia grade 2 and subsequent risk of cervical cancer: population based cohort study

Kathrine Dyhr Lycke,[1,2] Johnny Kahlert,[3] Lone Kjeld Petersen,[4,5] Rikke Kamp Damgaard,[1,2] Li C Cheung,[6] Patti E Gravitt,[7] Anne Hammer[1,2]

[1]Department of Obstetrics and Gynaecology, Gødstrup Hospital, Herning, Denmark
[2]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[3]Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark
[4]Department of Obstetrics and Gynaecology, Odense University Hospital, Odense, Denmark
[5]Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[6]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD, USA
[7]Centre for Global Health, National Cancer Institute, Rockville, MD, USA

Correspondence to: K D Lycke
katlyc@clin.au.dk
(ORCID 0000-0003-1657-8600)

Additional material is published online only. To view please visit the journal online.

Cite this as: *BMJ* 2023;383:e075925
http://dx.doi.org/10.1136/bmj-2023-075925

Accepted: 24 October 2023

ABSTRACT

OBJECTIVE

To describe the long term risk of cervical cancer in women with untreated (that is, undergoing active surveillance) or immediately treated cervical intraepithelial neoplasia grade 2 (CIN2).

DESIGN

Nationwide population based historical cohort study.

SETTING

Danish healthcare registries.

PARTICIPANTS

Women with CIN2 diagnosed in 1998-2020 and aged 18-40 years at diagnosis, who had either active surveillance or immediate treatment with large loop excision of the transformation zone (LLETZ). Women with a previous record of CIN2 or worse or LLETZ were excluded.

MAIN OUTCOME MEASURE

A Weibull survival model for interval censored time-to-event data was used to estimate the cumulative risk of cervical cancer. Inverse probability treatment weighting was used to adjust estimates for age, index cytology, calendar year, and region of residence.

RESULTS

The cohort included 27 524 women with CIN2, of whom 12 483 (45%) had active surveillance and 15 041 (55%) had immediate LLETZ. During follow-up, 104 cases of cervical cancer were identified—56 (54%) in the active surveillance group and 48 (46%) in the LLETZ group. The cumulative risk of cervical cancer was comparable across the two groups during the active surveillance period of two years. Thereafter, the risk increased in the active surveillance group, reaching 2.65% (95% confidence interval 2.07% to 3.23%) after 20 years, whereas it remained stable in the LLETZ group at 0.76% (0.58% to 0.95%).

CONCLUSIONS

Undergoing active surveillance for CIN2, thereby leaving the lesion untreated, was associated with increased long term risk of cervical cancer compared with immediate LLETZ. These findings show the importance of continued follow-up of women having active surveillance.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Several observational studies have shown that 50-60% of cases of cervical intraepithelial neoplasia grade 2 (CIN2) spontaneously regress within two years

This justifies leaving the lesion initially untreated instead of immediate treatment with large loop excision of the transformation zone (LLETZ)

Active surveillance has been implemented in many countries, but whether active surveillance is associated with increased risk of cervical cancer in the longer term is unclear

WHAT THIS STUDY ADDS

In this study on the long term risk of cervical cancer in women with CIN2, the absolute risk of cervical cancer after 20 years was low at 0.8-7%

Compared with immediate LLETZ, active surveillance was associated with a nearly fourfold higher risk of cervical cancer 20 years after diagnosis of CIN2

Introduction

Cervical intraepithelial neoplasia grade 2 (CIN2) is a precursor of cervical cancer and may progress to cancer if left untreated. Consequently, CIN2 has been the threshold for surgical excision—that is, large loop excision of the transformation zone (LLETZ). However, several studies have shown high spontaneous regression rates of CIN2 (50-60% within two years), suggesting a considerable risk of overtreatment if all women with CIN2 are treated with LLETZ.[1-3] This is concerning, particularly because LLETZ is associated with increased risk of preterm delivery in subsequent pregnancies.[4 5] As a result, many countries have implemented active surveillance as an option in younger women in whom CIN2 is diagnosed.[6]

However, this conservative approach has been introduced without any knowledge of its potential effect on the risk of cervical cancer. As active surveillance is based on follow-up with colposcopy and collection of colposcopy directed cervical biopsies, women having active surveillance may be at risk of missed prevalent disease, including cervical cancer.[7] In the longer term, acknowledgment that active surveillance implies that the lesion and underlying human papilloma virus infection is left untreated is important. Even if the histopathology indicates regression of CIN2, a potential risk of latent human papilloma virus infection may persist.[8-10] As women having LLETZ due to CIN2 or CIN3 have an increased risk of cervical cancer over their lifetime,[8 11] we hypothesise that women having active surveillance may have an even higher risk of cervical cancer. However, whether active surveillance of CIN2 is associated with increased risk of cervical cancer is unknown.

As active surveillance has only recently been implemented in many developed countries, cancer registry data have not sufficiently matured to evaluate this risk. Active surveillance has been an option in Denmark since 2013 and in some Danish regions since 1995.[12] Thus, using data from high quality and individual level Danish registries, we aimed to describe

the 20 year cumulative risk of cervical cancer in women having active surveillance for CIN2 compared with women treated with immediate LLETZ.

Methods

Setting

In Denmark, routine cervical cancer screening, including diagnostics and treatment of cervical precursor lesions, is free of charge for all women as the healthcare system is tax funded. Screening began in the 1960s for women aged 23-59 and was extended to women aged 60-64 in 2007.[12] Women with an abnormal screening result are recommended to have repeated testing or are referred to colposcopy, depending on the screening result. Abnormal areas are biopsied at colposcopy, but obtaining four biopsies from all women, regardless of the colposcopic finding, has been recommended since 2013.[12] Subsequent clinical management is determined by the histopathological diagnosis and the associated cytology.

Generally, until 2013, all women in whom CIN2 was diagnosed were recommended immediate LLETZ in Denmark. Thereafter, active surveillance has been an option to women of fertile age irrespective of parity, index cytology, or colposcopic findings. However, active surveillance has been an option in Central Region Denmark (comprising approximately 20% of the Danish population) since 1995.[13] Active surveillance consists of semi-annual follow-up visits with colposcopy, collection of cervical cytology, and multiple biopsies.[12] Women are recommended to have LLETZ in the case of progression or persistent disease after two years.

Study population and exposure

In Denmark, each resident is assigned a unique personal identification number at birth or immigration. This number ensures accurate and individual level linkage of the comprehensive and high quality Danish healthcare registries.[14] We used the Danish Pathology Registry to identify our study population. This registry is highly complete and holds information on all cytological and histological samples collected in public and private hospitals since 1998.[15] We did a nationwide population based historical cohort study on women aged 18-40 with a record of an incidental diagnosis of CIN2 on cervical biopsies from 1 January 1998 to 29 February 2020. We excluded women with a previous record of CIN2+, LLETZ, or hysterectomy (fig 1). Additionally, we excluded women with vulval or vaginal cancer before diagnosis of CIN2.

We classified the women into two groups on the basis of their first subsequent record in the Danish Pathology Registry within 10 months after diagnosis of CIN2. If the first follow-up record included cervical biopsy, cytology, or both, we classified women as having had active surveillance, whereas we classified women with a subsequent record of a LLETZ as having had immediate LLETZ. We considered women with no record within 10 months of CIN2 diagnosis to be non-compliant and excluded them from the analyses (fig 1). We chose a window of 10 months after incidental CIN2 diagnosis as this allowed for a diagnostic delay.[13]

Outcome

Our outcome was cervical cancer, which we identified through the Danish Pathology Registry, the Danish Cancer Registry, or both (see supplementary table A for definitions). The Danish Cancer Registry holds information on all incident cancers classified according to ICD-10 (the international classification of diseases, 10th revision).[16] This registry also contains information on cancer stage according to International Federation of Gynecology and Obstetrics (FIGO) classification.

Covariates

As potential confounding factors, we considered age, calendar year, and residential area (region of residence) at CIN2 diagnosis (see directed acyclic graph in supplementary figure A). These data came from the Civil Registration System and the Danish Pathology Registry.[14 15] Additionally, we considered the result of the index cytology as a confounder, and this information came from the Danish Pathology Registry. We defined the index cytology as the most recent cytology result within six months before and seven days after CIN2 diagnosis. We categorised the index cytology into the following groups: normal, low grade (atypical squamous cells of undetermined significance and low grade squamous intraepithelial lesion), high grade (atypical squamous cells—cannot exclude high grade squamous intraepithelial lesion, atypical glandular cells, high grade squamous intraepithelial lesion and carcinoma), and other/missing.

Statistical analyses

We followed women from diagnosis of CIN2 until cervical cancer, hysterectomy, emigration, death, or 31 December 2020, whichever occurred first. In the main analysis, we followed women from diagnosis of CIN2 regardless of whether women in the active surveillance group had a subsequent LLETZ (hereafter referred to as model 1). We did two ancillary analyses. In the first ancillary analysis, we censored women in the active surveillance group if a LLETZ was done during the active surveillance period of 28 months (model 2). We chose a window of 28 months as this allowed us to take into account LLETZ done because of persistent CIN2 after two years. In the second ancillary analysis, follow-up began after 28 months (model 3). This allowed us to evaluate the risk of cervical cancer in women without any excisional treatment during the surveillance period. Finally, we did a landmark analysis on model 1 with an intercept at 28 months. We reported results overall and stratified by age at diagnosis of CIN2 (<30, ≥30 years), index cytology (high grade, normal/low grade/other/missing), and calendar year (1998-2006, 2007-12, 2013-20).

To estimate the cumulative risk of cervical cancer, we fitted a Weibull model for interval censored time-to-event data. As cervical cancer often presents without

doi: 10.1136/bmj-2023-075925 | *BMJ* 2023;383:e075925 | the bmj

symptoms, using standard methods that equate onset with clinical diagnosis will lead to biased estimates that depend on the assessment time.[17 18] Instead, cancer onset is treated as occurring in the interval between the last disease-free histological record (cervical biopsies, endocervical curettage, LLETZ, and hysterectomy) and the time of cancer diagnosis. Individuals without a cancer diagnosis are right censored at the time of the last disease-free histological record. Observations were also right censored when LLETZ censoring (model 2), emigration, death, or a hysterectomy was recorded before an incident cervical cancer. The Weibull model, which was proposed by Armitage and Doll as a model for carcinogenesis, has been applied in a wide range of studies of cancer development[19]; it provides more flexibility and precision in risk estimates than do the non-parametric (Kaplan-Meier estimator-like) analogues that incorporate interval censored data, such as the Turnbull estimator.[17 20]

We visually compared the graphical presentations of the Weibull and Turnbull cumulative risk estimates for cervical cancer to ensure that the Weibull model was a good fit for the data (supplementary figure B). Turnbull estimates are generally considered to be unbiased, although less reliable at specific time points of interest if large jumps in the cumulative risk estimates are present.[17]

We used stabilised inverse probability treatment weighting to balance covariates across the two groups (active surveillance and immediate LLETZ) and to adjust for potential confounders. As previously described, we included the following covariates in the propensity model: age, index cytology, calendar year, and region of residence, together with first order interactions. Furthermore, we applied weight trimming to reduce the importance of large weights[21]; these were trimmed to the 99th centile. We considered a covariate to be well balanced if the standardised difference was <0.1. We managed data and did statistical analyses on the remote servers of the Danish Health Data Authority, using SAS (version 9.4).

Patient and public involvement

No patients or members of the public were involved in determining the research question, study design, analyses, interpretation of data, or revision of the manuscript. The primary barrier was the nature of this study, in which we used pseudonymised registry data. However, our group has previously done a qualitative study in women with CIN2 who had active surveillance, which showed that women are concerned about the risk of cervical cancer.[22]

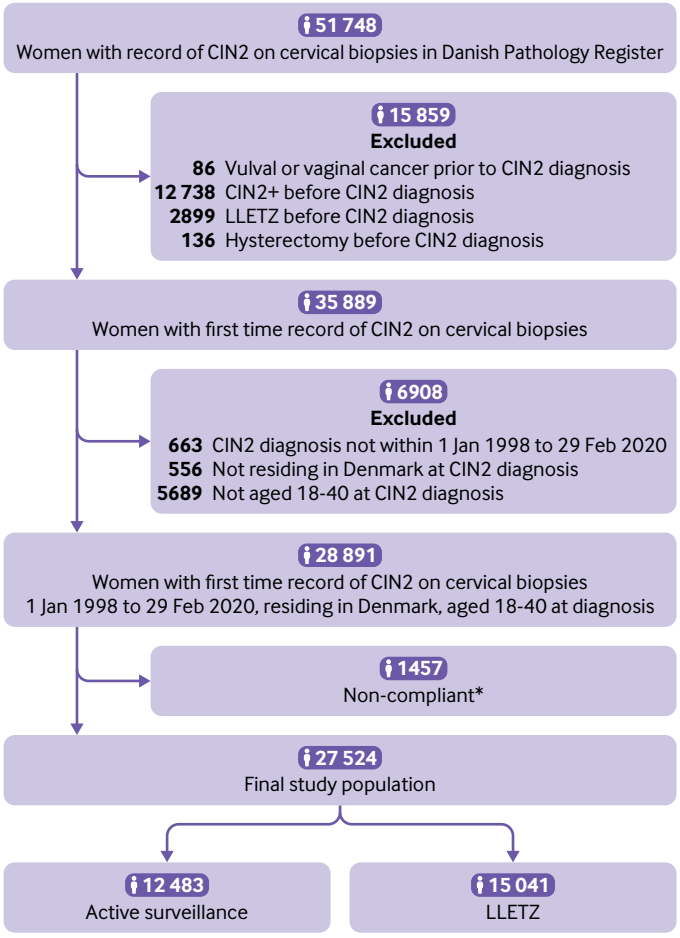


Fig 1 | Flowchart of study population. CIN2=cervical intraepithelial neoplasia grade 2; LLETZ=large loop excision of transformation zone

Results

We identified 28 891 women with an incidental diagnosis of CIN2 in the study period (fig 1). After exclusion of non-compliant women (baseline characteristics of the non-compliant women are shown in supplementary table B), we included 27 524 women, of whom 12 483 (45%) had active surveillance and 15 041 (55%) had an immediate LLETZ (table 1). From 1998 to 2012 most women had immediate LLETZ (66%), whereas after 2013 most had active surveillance (68%). Women in the active surveillance group were younger (median age 26 (interquartile range 23-30)) than women treated with immediate LLETZ (median age 30 (26-35)). Overall, most women had an abnormal index cytology (85%) with high grade cytology being predominant in both groups (46% and 54%, respectively).

The comparability between the two groups increased after application of stabilised inverse probability treatment weighting. Before the application of weighting, the standardised differences ranged from 0.01 to 0.67. After weighting, all standard differences were <0.1, so we considered the covariates to be well balanced between the two groups (table 2). Histograms of the distribution of propensity scores before and after adjustment are shown in supplementary figure C.

We identified 104 cases of cervical cancer; 56 (54%) cases were diagnosed in the active surveillance group and 48 (46%) cases in the immediate LLETZ group. In the main analysis, the adjusted cumulative risk of cervical cancer after two years was 0.56% (95% confidence interval 0.40% to 0.71%) in the active surveillance group and 0.37% (0.31% to 0.44%) in the

Table 1 | Baseline characteristics of women with record of incidental cervical intraepithelial neoplasia grade 2 in Denmark during 1998-2020. Values are numbers (percentages)

Characteristics	Active surveillance (n=12 483)	LLETZ (n=15 041)	Total (n=27 524)
Age group, years:			
18-22	1304 (10.4)	822 (5.5)	2126 (7.7)
23-29	7518 (60.2)	6388 (42.5)	13 906 (50.5)
30-40	3661 (29.3)	7831 (52.1)	11 492 (41.8)
Index cytology:			
Normal	1403 (11.2)	1083 (7.2)	2486 (9.0)
Low grade*	4450 (35.6)	4796 (31.9)	9246 (33.6)
High grade†	5769 (46.2)	8154 (54.2)	13 923 (50.6)
Other/missing	861 (6.9)	1008 (6.7)	1869 (6.8)
Year of diagnosis:			
1998-2006	2579 (20.7)	5875 (39.1)	8454 (30.7)
2007-12	3647 (29.2)	6209 (41.3)	9856 (35.8)
2013-20	6257 (50.1)	2957 (19.7)	9214 (33.5)
Region of residence:			
Capital	3511 (28.1)	6542 (43.5)	10 053 (36.5)
Central Region	4890 (39.2)	1838 (12.2)	6728 (24.4)
Northern Region	1285 (10.3)	1726 (11.5)	3011 (10.9)
Zealand	719 (5.8)	1807 (12.0)	2526 (9.2)
Southern Region	2078 (16.6)	3128 (20.8)	5206 (18.9)

LLETZ=large loop excision of the transformation zone.
*Includes atypical squamous cells of undetermined significance and low grade squamous intraepithelial lesion.
†Includes atypical squamous cells—cannot exclude high grade squamous intraepithelial lesion (HSIL), atypical glandular cells, HSIL, adenocarcinoma in situ, and carcinoma.

LLETZ group (table 3; fig 2). Thereafter, the cumulative risk increased for women in the active surveillance group but remained relatively stable for women who had immediate LLETZ (see also the landmark analysis in supplementary figure D). After 20 years of follow-up, the cumulative risk of cervical cancer was nearly fourfold higher for women in the active surveillance group (2.65%, 2.07% to 3.23%) compared with women in the immediate LLETZ group (0.76%, 0.58% to 0.95%). The distribution of attrition was comparable between active surveillance and immediate LLETZ (supplementary table C), and only a small number of women were lost to follow-up (3.1% in both groups). Median follow-up was shorter in the active surveillance group (12 (interquartile range 6-29) months) than in the immediate LLETZ group (2 (1-22) months). Additionally, the number of histological records after 28 months was comparable between the two groups (supplementary table C).

Overall, we observed no difference in the FIGO cancer stage between the two groups, and fewer than five of the registered cases were ≥2B (supplementary table D).

Table 2 | Characteristics of women with cervical intraepithelial neoplasia grade 2 undergoing active surveillance or immediate large loop excision of transformation zone (LLETZ) for crude cohort, after stabilised inverse probability treatment weighting (SIPTW), and after SIPTW and weight trimming. Values are numbers (percentages) unless stated otherwise

	Overall crude cohort			Propensity score weighted cohort (after SIPTW)			Propensity score weighted cohort (after SIPTW and weight trimming)		
Characteristics	Active surveillance (n=12 483)	LLETZ (n=15 041)	SD	Active surveillance (n=12 484)	LLETZ (n=14 987)	SD	Active surveillance (n=12 407)	LLETZ (n=14 402)	SD
Age group, years:									
18-22	1304 (10.4)	822 (5.5)	0.18	976 (7.8)	1141 (7.6)	0.01	976 (7.9)	1001 (7.0)	0.03
23-29	7518 (60.2)	6388 (42.5)	0.36	6298 (50.4)	7562 (50.5)	0.01	6295 (50.7)	7117 (49.4)	0.03
30-40	3661 (29.3)	7831 (52.1)	0.47	5209 (41.7)	6284 (41.9)	0.01	5136 (41.4)	6284 (43.6)	0.04
Index cytology:									
Normal	1403 (11.2)	1083 (7.2)	0.14	6318 (50.6)	7622 (50.9)	0.01	1139 (9.2)	1233 (8.6)	0.02
Low grade*	4450 (35.6)	4796 (31.9)	0.08	4183 (33.5)	5016 (33.5)	0.01	4160 (33.5)	4685 (32.5)	0.02
High grade†	5769 (46.2)	8154 (54.2)	0.16	1141 (9.1)	1319 (8.8)	0.01	6268 (50.5)	7485 (52.0)	0.03
Other/missing	861 (6.9)	1008 (6.7)	0.01	841 (6.7)	1030 (6.9)	0.01	841 (6.8)	998 (6.9)	0.01
Year of diagnosis:									
1998-2006	2579 (20.7)	5875 (39.1)	0.41	3855 (30.9)	4618 (30.8)	0.01	3855 (31.1)	4618 (32.1)	0.02
2007-12	3647 (29.2)	6209 (41.3)	0.25	4451 (35.7)	5412 (36.1)	0.01	4374 (35.3)	5267 (36.6)	0.03
2013-20	6257 (50.1)	2957 (19.7)	0.67	4178 (33.5)	4957 (33.1)	0.01	4178 (33.7)	4517 (31.4)	0.05
Region of residence:									
Capital	3511 (28.1)	6542 (43.5)	0.32	4549 (36.4)	5547 (37.0)	0.01	4549 (36.7)	5535 (38.4)	0.04
Central Region	4890 (39.2)	1838 (12.2)	0.65	3052 (24.4)	3666 (24.5)	0.01	3052 (24.6)	3132 (21.7)	0.07
Northern Region	1285 (10.3)	1726 (11.5)	0.04	1382 (11.1)	1576 (10.5)	0.02	1382 (11.1)	1543 (10.7)	0.01
Zealand	719 (5.8)	1807 (12.0)	0.22	1144 (9.2)	1383 (9.2)	0.01	1068 (8.6)	1383 (9.6)	0.03
Southern Region	2078 (16.6)	3128 (20.8)	0.11	2357 (18.9)	2814 (18.8)	0.01	2357 (19.0)	2809 (19.5)	0.01

SD=standardised difference.
*Includes atypical squamous cells of undetermined significance and low grade squamous intraepithelial lesion.
†Includes atypical squamous cells—cannot exclude high grade squamous intraepithelial lesion (HSIL), atypical glandular cells, HSIL, adenocarcinoma in situ, and carcinoma.

Table 3 | Adjusted cumulative risk of cervical cancer among women with cervical intraepithelial neoplasia grade 2 (CIN2) undergoing active surveillance or immediate large loop excision of transformation zone (LLETZ), depicting estimates for model 1 and 2. Values are percentages with 95% confidence intervals

Model	1 year	2 years	5 years	10 years	15 years	20 years
Model 1*						
Crude:						
Active surveillance	0.27 (0.19 to 0.35)	0.46 (0.35 to 0.57)	0.94 (0.75 to 1.13)	1.61 (1.29 to 1.93)	2.20 (1.72 to 2.69)	2.75 (2.10 to 3.40)
LLETZ	0.36 (0.32 to 0.40)	0.44 (0.38 to 0.50)	0.59 (0.49 to 0.68)	0.72 (0.59 to 0.85)	0.82 (0.66 to 0.97)	0.89 (0.71 to 1.07)
Adjusted:						
Active surveillance	0.35 (0.23 to 0.47)	0.56 (0.40 to 0.71)	1.04 (0.81 to 1.26)	1.66 (1.33 to 1.99)	2.18 (1.73 to 2.64)	2.65 (2.07 to 3.23)
LLETZ	0.30 (0.25 to 0.35)	0.37 (0.31 to 0.44)	0.50 (0.40 to 0.59)	0.62 (0.48 to 0.75)	0.70 (0.54 to 0.86)	0.76 (0.58 to 0.95)
Model 2†						
Crude:						
Active surveillance	0.17 (0.09 to 0.25)	0.33 (0.23 to 0.44)	0.79 (0.62 to 0.97)	1.52 (1.13 to 1.92)	2.23 (1.51 to 2.94)	2.91 (1.82 to 4.01)
LLETZ	0.36 (0.32 to 0.40)	0.44 (0.38 to 0.50)	0.59 (0.49 to 0.68)	0.72 (0.59 to 0.85)	0.82 (0.66 to 0.97)	0.89 (0.71 to 1.07)
Adjusted:						
Active surveillance	0.24 (0.12 to 0.36)	0.41 (0.26 to 0.56)	0.83 (0.63 to 1.04)	1.42 (1.05 to 1.80)	1.94 (1.32 to 2.56)	2.42 (1.51 to 3.32)
LLETZ	0.30 (0.25 to 0.35)	0.37 (0.31 to 0.44)	0.50 (0.40 to 0.59)	0.62 (0.48 to 0.75)	0.70 (0.54 to 0.86)	0.76 (0.58 to 0.95)

*Follow-up from date of CIN2 diagnosis.
†Follow-up from date of CIN2 diagnosis. Women in active surveillance group who were treated with LLETZ owing to progression or persistent CIN2 within 28 months of follow-up were censored at date of LLETZ.

In the excluded non-compliant women, we identified seven cases of cervical cancer, corresponding to 0.5% (supplementary table B).

When we stratified by age at diagnosis of CIN2, most cases of cervical cancer (n=71; 68%) were diagnosed in women aged ≥30 years (table 4; fig 3). This corresponded to a threefold to fourfold higher cumulative risk of cervical cancer after the first two years for both groups. After 20 years, the cumulative risk in the active surveillance group was threefold higher in women ≥30 years (5.30%, 3.91% to 6.69%) compared with women <30 years (1.52%, 0.92% to 2.12%). For women in the LLETZ group, the risk was twofold higher in women aged ≥30 years (1.27%, 0.94% to 1.60%) than in women <30 years (0.55%, 0.34% to 0.76%) after 20 years of follow-up.

With respect to index cytology, women with high grade index cytology had higher risk of cervical cancer than did women with normal, low grade, or missing/other index cytology (table 4; supplementary figure E). After two years, the risk was nearly doubled for women with high grade index cytology in both groups. After 20 years, the cumulative risk in the active surveillance group was slightly higher for women with high grade index cytology (2.96%, 2.06% to 3.86%) than in women with non-high grade index cytology (2.41%, 1.50% to 3.33%). Similarly, for the LLETZ group, the risk was slightly higher among women with high grade compared with non-high grade index cytology—1.23% (0.95% to 1.50%) and 0.59% (0.43% to 0.76%), respectively.

When we stratified by calendar year of CIN2 diagnosis, the risk of cervical cancer was higher in the active surveillance group than in the immediate LLETZ group for women with CIN2 diagnosed in 1998-2006 and 2013-20 (supplementary figure F). However, for women with CIN2 diagnosed in 2007-12, the risk of cervical cancer was comparable between women having active surveillance and those having immediate LLETZ.

In our ancillary analysis (model 2), in which we censored at time of LLETZ for women having active surveillance (n=4483; 35.9%), we found similar results with increased risk of cervical cancer for women having active surveillance compared with those having immediate LLETZ (table 3; fig 2). The difference between the two groups was slightly lower compared with model 1. After 20 years, the cumulative risk was 2.42% (1.51% to 3.32%) for women in the active surveillance group, which was more than threefold higher than the observed risk for the LLETZ group (0.76%, 0.58% to 0.95%) (table 3).

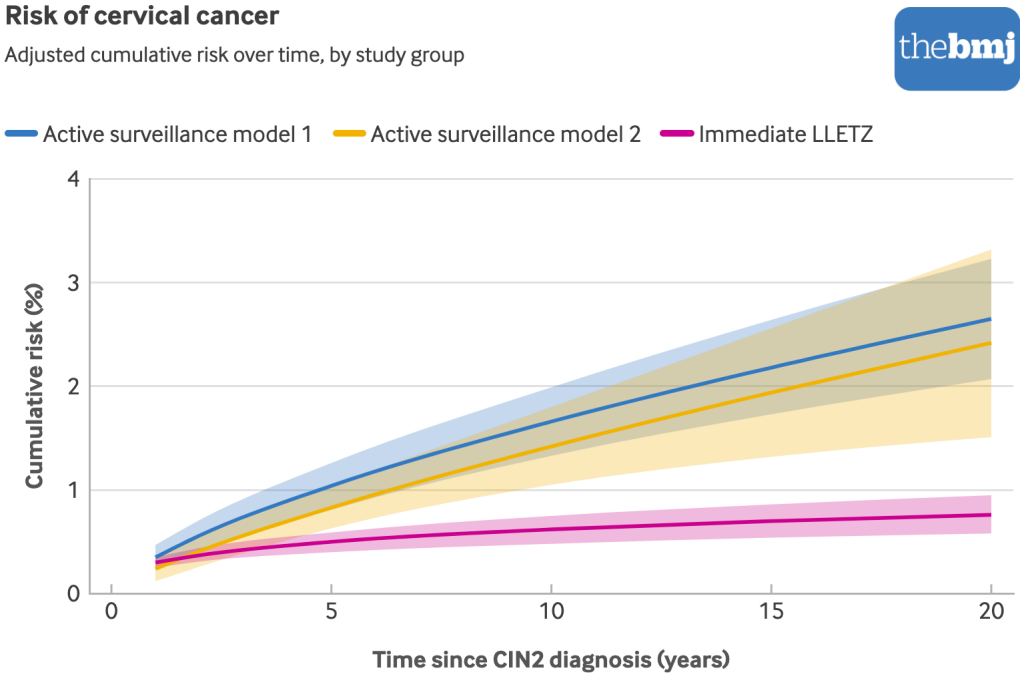
Finally, in the analysis in which we started follow-up after 28 months (model 3), we found similar results with a considerably higher risk of cervical cancer in women who had active surveillance for CIN2 compared with those who had immediate LLETZ (supplementary figure G and table E). After 15 years of follow-up, the cumulative risk was 3.83% (3.24% to 4.42%) for women having active surveillance and 0.69% (0.46% to 0.93%) for those having LLETZ.

Discussion

In this population based study of 27 524 women with CIN2, we found that the absolute risk of cervical cancer was low. Although we observed no major difference in risk during the two year active surveillance period, the cumulative risk was nearly fourfold higher in women who had active surveillance compared with those who had LLETZ after 20 years of follow-up. The increased risk in the active surveillance group was primarily driven by women aged ≥30 years. Our findings are important for clinical counselling of women with CIN2 and suggest a need for increased follow-up in women with a history of active surveillance.

Comparison with other studies

In this study, the two year cumulative risk of cervical cancer was 0.56% (0.40% to 0.71%) in women having active surveillance. This is slightly higher than the pooled estimates from two recent systematic reviews and meta-analyses in which cancer was diagnosed in 0.33-0.47% of women within two years.[12] However,



Article DOI: 10.1136/BMJ-2023-075925

Fig 2 | Cumulative risk of cervical cancer among women with cervical intraepithelial neoplasia grade 2 (CIN2) having active surveillance or immediate large loop excision of transformation zone (LLETZ). Model 1=follow-up from date of CIN2 diagnosis; model 2=follow-up from date of CIN2 diagnosis. Women in active surveillance group who were treated with LLETZ within 28 months of follow-up were censored at date of LLETZ. An interactive version of this graphic is available at https://public.flourish.studio/visualisation/15560724/

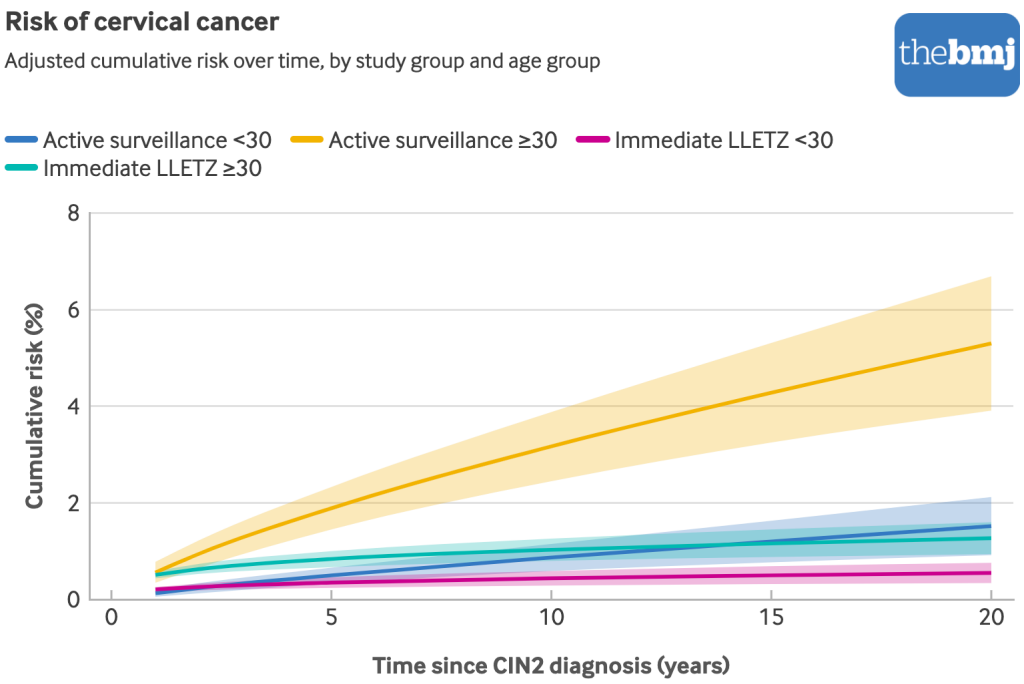
most of the studies in the meta-analyses included women with lower risk of progression than our study population; these studies were often restricted to women younger than 25 or 30 years, women with small lesions (up to two quadrants), and/or women with low grade index cytology.

Cases of cervical cancer diagnosed within the two year surveillance period may represent missed prevalent disease due to sampling error or misinterpretation of the histological specimen. Although the risk of sampling error is higher in cervical biopsies than in LLETZ,[7 23] we found that the risk of cervical cancer was comparable between the two groups in the first two years of follow-up. Thus, the risk of sampling error for women under active surveillance seems limited, possibly owing to the repeated cervical biopsies. Therefore, active surveillance of CIN2 for up to two years seems to be justified, which is also supported by previous studies in which the spontaneous regression rates of CIN2 were 50-60% within the first two years.[1 23]

Table 4 | Adjusted cumulative risk* of cervical cancer stratified by age (<30, ≤30 years) and index cytology (high grade, non-high grade) in women with cervical intraepithelial neoplasia grade 2 (CIN2) undergoing active surveillance or immediate large loop excision of transformation zone LLETZ. Values are percentages with 95% confidence intervals

Group	No of cases	1 year	2 years	5 years	10 years	15 years	20 years
Age at CIN2 diagnosis							
Active surveillance:							
<30 years	20	0.13 (0.06 to 0.21)	0.24 (0.13 to 0.34)	0.50 (0.33 to 0.66)	0.87 (0.59 to 1.15)	1.20 (0.77 to 1.63)	1.52 (0.92 to 2.12)
≥30 years	36	0.56 (0.35 to 0.78)	0.95 (0.66 to 1.23)	1.89 (1.45 to 2.33)	3.17 (2.45 to 3.88)	4.28 (3.25 to 5.31)	5.30 (3.91 to 6.69)
LLETZ:							
<30 years	13	0.21 (0.15 to 0.26)	0.26 (0.19 to 0.33)	0.35 (0.24 to 0.46)	0.44 (0.29 to 0.59)	0.50 (0.32 to 0.69)	0.55 (0.34 to 0.76)
≥30 years	35	0.51 (0.44 to 0.59)	0.63 (0.53 to 0.74)	0.84 (0.67 to 1.00)	1.03 (0.80 to 1.26)	1.16 (0.88 to 1.45)	1.27 (0.94 to 1.60)
Index cytology							
Active surveillance:							
Non-high grade†	26	0.23 (0.11 to 0.35)	0.40 (0.24 to 0.56)	0.82 (0.59 to 1.05)	1.41 (1.00 to 1.82)	1.93 (1.29 to 2.57)	2.41 (1.50 to 3.33)
High grade	30	0.32 (0.20 to 0.43)	0.53 (0.36 to 0.70)	1.06 (0.77 to 1.35)	1.77 (1.28 to 2.26)	2.39 (1.70 to 3.08)	2.96 (2.06 to 3.86)
LLETZ:							
Non-high grade†	15	0.24 (0.19 to 0.29)	0.30 (0.24 to 0.36)	0.39 (0.30 to 0.48)	0.48 (0.36 to 0.60)	0.54 (0.40 to 0.69)	0.59 (0.43 to 0.76)
High grade	33	0.48 (0.41 to 0.54)	0.59 (0.50 to 0.69)	0.79 (0.65 to 0.94)	0.99 (0.79 to 1.18)	1.12 (0.88 to 1.36)	1.23 (0.95 to 1.50)

*All estimates are based on model 1 and are adjusted.
†Includes normal, low grade, missing/other.



Article DOI: 10.1136/BMJ-2023-075925

Fig 3 | Cumulative risk of cervical cancer among women with cervical intraepithelial neoplasia grade 2 (CIN2) having active surveillance or immediate large loop excision of transformation zone (LLETZ), stratified by age (<30, ≤30). Graph is based on model 1. An interactive version of this graphic is available at https://public.flourish.studio/visualisation/15561284/

On the other hand, up to 40-50% of women progress or have persistent disease within two years and are in need of excisional treatment.[12] Risk stratification of women with CIN2 to identify those at highest risk of progression is therefore highly clinically relevant. Recent studies have suggested that human papilloma virus genotyping and methylation markers may be useful for such stratification.[24 25]

Although the risk of cervical cancer was comparable within the first two years, the risk thereafter increased in the active surveillance group. After 20 years, the risk was approximately fourfold higher in the active surveillance group (2.65%, 2.07% to 3.23%), whereas it remained stable in the LLETZ group (0.76%, 0.58% to 0.95%). The observed estimate for the LLETZ group is comparable to those in previous studies on long term risk of cervical cancer in women treated with LLETZ—that is, the 20-25 year risk is approximately 1%.[8 11 26 27] Although the long term risk of cancer after LLETZ is well investigated, this study investigated the risk in women managed with active surveillance for CIN2 with follow-up for up to 20 years.

We found the highest risk of cancer in women aged ≥30 years having active surveillance (table 4; fig 3). Although Danish women of fertile age are offered active surveillance, many countries restrict active surveillance to women aged <25-30 years.[6] The age restriction seems justified on the basis of our results. However, consideration that this age restriction (<25-30 years) may not have the desired effect on reducing the number of preterm deliveries associated with LLETZ is important, as women in most developed countries give birth well into their 30s.[4 28]

One explanation for the higher long term risk of cervical cancer in women having active surveillance could be that the lesion and underlying human papilloma virus infection are left untreated in case of regression. The human papilloma virus infection may therefore persist as detectable or undetectable latent infection, even in the absence of colposcopic abnormalities. Thus, regressed CIN2 may be only a temporary state in some women at high risk. For example, a previous study on CIN2 has shown that women experiencing initial regression have a threefold higher risk of recurrent high grade cytology (high grade squamous intraepithelial lesion or atypical squamous cells—cannot exclude high grade squamous intraepithelial lesion) or histology (CIN2 or CIN3) within four years compared with women having a LLETZ.[29] These findings support the hypothesis that human papilloma virus may be able to establish latency on lesion regression with subsequent risk of viral reactivation during periods of immune incompetence or increasing age.[8-10] However, no evidence suggests a higher risk of CIN3+ in women with reactivated latent human papilloma virus infections compared with apparent incident detection of human papilloma virus.[8 30]

Another possible explanation for the observed difference in risk of cancer between the groups could

be differences in frequency and type of histological sampling during follow-up—that is, surveillance and detection bias. During the first two years, women in the active surveillance group may have repeated histological sampling. However, as most women experience regression or progression within the first year after diagnosis of CIN2, only a few women have cervical biopsies collected multiple times.[3 24] Active surveillance is completed by either exit to repeated cytological testing for women with regression or LLETZ in the case of persistent CIN2 or progression.[12] The frequency and type of histological sampling is thereafter dependent on the result of cytology and cone specimen. For women treated with LLETZ, the standard care after LLETZ was annual cytology, cervical biopsies, or both (depending on the margin status of the cone specimen) from 1998 to 2012, whereas standard care after 2013 was human papilloma virus and cytology co-testing.[12] Additionally, when we evaluated the number of histological records in both groups (supplementary table C), we observed no difference in the number of histological records after ≥28 months after diagnosis of CIN2. Of note, a minor fraction of the immediate LLETZ group were treated with repeated LLETZ (n=1026; 6.8%).

Policy implications

Our findings suggest the need for a decision making process for treatment of CIN2 based on age and reproductive desire. Active surveillance for two years seems to be safe in terms of risk of cancer for women who are planning pregnancy (both younger and older women). However, once women having active surveillance have completed their planned pregnancies, a shared discussion of long term risk of cervical cancer may be warranted, possibly including an offer of LLETZ after completion of child bearing. Our results may imply that the threshold for LLETZ should be lower in the case of suspected recurrent disease in women with a history of active surveillance for CIN2. Most importantly, women should be properly informed about the risks and benefits of active surveillance, especially considering that active surveillance is associated with an increased level of anxiety and concerns about disease progression.[22 31]

Strengths and limitations of study

The strengths of this study include its population based design, with the use of individual level data from high quality nationwide registries with virtually complete follow-up.[14-16] Also, as all women of fertile age are eligible for active surveillance in Denmark, we were able to do analyses stratified by age and index cytology.

This study also has some limitations. The diagnosis of CIN2 is well known to have a high intra-observer and inter-observer variation.[32 33] Thus, we cannot rule out misclassification of CIN2, but we do not expect differential misclassification. Furthermore, if women treated with immediate LLETZ were more likely to be misclassified as CIN3, this would result in bias towards the null. Also, we used the diagnostic information that was available to the gynaecologist. Secondly, confounding by indication may be present in our study. However, active surveillance is offered to all women of fertile age in Denmark, regardless of family planning, lesion size, colposcopic impression, smoking, and so on. Thus, we expect limited impact of these variables on our findings. As these variables are not recorded in the registries, we were not able to assess this further. However, we considered index cytology as a proxy for lesion size, as the likelihood of small lesions is higher in the case of low grade cytology.[34] Thirdly, we had no information on socioeconomic status. A higher socioeconomic status is positively correlated with participation in screening[35 36]; however, whether socioeconomic status is associated with active surveillance for CIN2 is unknown, although we note that all women in Denmark have free access to management and treatment of CIN. Thus, we expect limited effect of potential differences in socioeconomic status. Fourthly, among women having LLETZ, the risk of cervical cancer differs depending on the margin status of the cone specimen.[26 37] Unfortunately, we had no information on margin status. Fifthly, we had no information on the number of biopsies in the active surveillance group; the number of biopsies is positively correlated with the CIN2+ detection rate.[38] Finally, our use of a 10 month window for assessment of exposure and start of follow-up at diagnosis of CIN2 (models 1 and 2) may have introduced immortal time bias. However, as fewer than five deaths occurred during this assessment window, we consider this bias to be practically non-existent.

Conclusion

Overall, the absolute risk of cervical cancer was low in women with a history of CIN2. Within the first years of follow-up, the risk of cervical cancer was comparable between active surveillance and LLETZ. Thereafter, active surveillance was associated with increased risk of cervical cancer compared with immediate LLETZ. After 20 years, the risk was nearly fourfold higher. Our findings are important for future guidelines on management of CIN2 and clinical counselling of women with a diagnosis of CIN2.

Contributors: KDL, JK, LKP, RKD, and AH conceived and designed the study. KDL, JK, and AH obtained the data from the Danish Health Data Authority. KDL, JK, and AH verified the data. JK and KDL did the analyses. LCC co-supervised the analyses. LKP, RKD, LCC, PEG, and AH critically discussed the analyses and results. KDL and AH drafted the manuscript. All authors critically revised the paper and approved the final version. KDL is the guarantor. The corresponding affirms that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding: The study was funded by the Danish Cancer Society (R248-A14476), Central Region Denmark, Carpenter Axel Kastrup-Nielsen's Memorial Fund, Manufacturer Einar Willumsen's Memorial Fund, and Merchant AV Lykfeldt's Grant. None of the funding sources had any influence on the study design, analyses, interpretation of data, writing the paper, or the decision to submit the paper.

Competing interests: All authors have completed the ICMJE uniform disclosure form at https://www.icmje.org/disclosure-of-interest/ and declare: support from the Danish Cancer Society, Central Region Denmark, Carpenter Axel Kastrup-Nielsen's Memorial Fund, Manufacturer Einar Willumsen's Memorial Fund, and Merchant AV

Lykfeldt's Grant; KDL has received a speaker's fee from AstraZeneca; RKD and AH have received reagents at reduced cost from Roche Denmark; LP has received a speaker's fee from MSD; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: According to Danish legislation, approval from the ethics committee is not required for registry based research. The study was reported to the Danish Data Protection Agency through registration at Aarhus University (2016-051-000001, sequential number 1648), Central Region Denmark (1-16-02-367-21), and Danish Health Data Authority (FSEID-00005496).

Data sharing: This study was conducted on the remote servers of the Danish Health Data Authority. Owing to Danish legislation, individual level data cannot be shared by the authors, and in cell counts actual numbers less than five cannot be reported. Data can be accessed after application to the Danish Health Data Authority.

The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Dissemination to participants and related patient and public communities: The findings of this study will be shared with clinicians and advocacy groups via the website of the authors' institutions and on social media.

Provenance and peer review: Not commissioned; externally peer reviewed.

1 Tainio K, Athanasiou A, Tikkinen KAO, et al. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta-analysis. *BMJ* 2018;360:k499. doi:10.1136/bmj.k499
2 Loopik DL, Bentley HA, Eijgenraam MN, IntHout J, Bekkers RLM, Bentley JR. The Natural History of Cervical Intraepithelial Neoplasia Grades 1, 2, and 3: A Systematic Review and Meta-analysis. *J Low Genit Tract Dis* 2021;25:221-31. doi:10.1097/LGT.0000000000000604
3 Lycke KD, Kahlert J, Damgaard RK, et al. Clinical course of cervical intraepithelial neoplasia grade 2: a population-based cohort study. *Am J Obstet Gynecol* 2023;S0002-9378(23)00542-2. doi:10.1016/j.ajog.2023.08.008
4 Kyrgiou M, Athanasiou A, Paraskevaidi M, et al. Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. *BMJ* 2016;354:i3633. doi:10.1136/bmj.i3633
5 Athanasiou A, Veroniki AA, Efthimiou O, et al. Comparative effectiveness and risk of preterm birth of local treatments for cervical intraepithelial neoplasia and stage IA1 cervical cancer: a systematic review and network meta-analysis. *Lancet Oncol* 2022;23:1097-108. doi:10.1016/S1470-2045(22)00334-5
6 Lycke KD, Petersen LK, Gravitt PE, Hammer A. Known Benefits and Unknown Risks of Active Surveillance of Cervical Intraepithelial Neoplasia Grade 2. *Obstet Gynecol* 2022;139:680-6. doi:10.1097/AOG.0000000000004705
7 Stoler MH, Vichnin MD, Ferenczy A, et al, FUTURE I, II and III Investigators. The accuracy of colposcopic biopsy: analyses from the placebo arm of the Gardasil clinical trials. *Int J Cancer* 2011;128:1354-62. doi:10.1002/ijc.25470
8 Gravitt PE, Winer RL. Natural History of HPV Infection across the Lifespan: Role of Viral Latency. *Viruses* 2017;9:267. doi:10.3390/v9100267
9 Leonard SM, Pereira M, Roberts S, et al. Evidence of disrupted high-risk human papillomavirus DNA in morphologically normal cervices of older women. *Sci Rep* 2016;6:20847. doi:10.1038/srep20847
10 Hammer A, de Koning MNC, Blaakaer J, et al. Whole tissue cervical mapping of HPV infection: Molecular evidence for focal latent HPV infection in humans. *Papillomavirus Res* 2019;7:82-7. doi:10.1016/j.pvr.2019.02.004
11 Kalliala I, Athanasiou A, Veroniki AA, et al. Incidence and mortality from cervical cancer and other malignancies after treatment of cervical intraepithelial neoplasia: a systematic review and meta-analysis of the literature. *Ann Oncol* 2020;31:213-27. doi:10.1016/j.annonc.2019.11.004
12 Danish Society of Obstetrics and Gynecology. Udredning, behandling og kontrol af cervikal dysplasia. 2012. http://gynobsguideline.dk/hindsgavl/Cervixdysplasi2012.pdf.
13 Skorstengaard M, Lynge E, Suhr J, Napolitano G. Conservative management of women with cervical intraepithelial neoplasia grade 2 in Denmark: a cohort study. *BJOG* 2020;127:729-36. doi:10.1111/1471-0528.16081
14 Pedersen CB. The Danish Civil Registration System. *Scand J Public Health* 2011;39(Suppl):22-5. doi:10.1177/1403494810387965
15 Bjerregaard B, Larsen OB. The Danish Pathology Register. *Scand J Public Health* 2011;39(Suppl):72-4. doi:10.1177/1403494810393563
16 Gjerstorff ML. The Danish Cancer Registry. *Scand J Public Health* 2011;39(Suppl):42-5. doi:10.1177/1403494810393562
17 Landy R, Cheung LC, Schiffman M, et al. Challenges in risk estimation using routinely collected clinical data: The example of estimating cervical cancer risks from electronic health-records. *Prev Med* 2018;111:429-35. doi:10.1016/j.ypmed.2017.12.004
18 Cheung LC, Pan Q, Hyun N, et al. Mixture models for undiagnosed prevalent disease and interval-censored incident disease: applications to a cohort assembled from electronic health records. *Stat Med* 2017;36:3583-95. doi:10.1002/sim.7380
19 Kopp-Schneider A. Carcinogenesis models for risk assessment. *Stat Methods Med Res* 1997;6:317-40. doi:10.1177/096228029700600403
20 Turnbull B. The Empirical Distribution Function with Arbitrarily Grouped, Censored and Truncated Data. *J R Stat Soc B* 1976;38:290-5. doi:10.1111/j.2517-6161.1976.tb01597.x.
21 Kahlert J, Gribsholt SB, Gammelager H, Dekkers OM, Luta G. Control of confounding in the analysis phase - an overview for clinicians. *Clin Epidemiol* 2017;9:195-204. doi:10.2147/CLEP.S129886
22 Hansen J, Kirkegaard P, Folmann B, Bungum HF, Hammer A. "I feel reassured, but there is no guarantee." How do women with a future childbearing desire respond to active surveillance of cervical intraepithelial neoplasia grade 2? A qualitative study. *Acta Obstet Gynecol Scand* 2022;101:616-23. doi:10.1111/aogs.14354
23 Booth BB, Petersen LK, Blaakaer J, et al. Accuracy of colposcopy-directed biopsy vs dynamic spectral imaging directed biopsy in correctly identifying the grade of cervical dysplasia in women undergoing conization: A methodological study. *Acta Obstet Gynecol Scand* 2020;99:1064-70. doi:10.1111/aogs.13832
24 Kylebäck K, Ekeryd-Andalen A, Greppe C, Björkenfeldt Havel C, Zhang C, Strander B. Active expectancy as alternative to treatment for cervical intraepithelial neoplasia grade 2 in women aged 25 to 30 years: ExCIN2-a prospective clinical multicenter cohort study. *Am J Obstet Gynecol* 2022;227:742.e1-11. doi:10.1016/j.ajog.2022.06.051
25 Louvanto K, Aro K, Nedjai B, et al. Methylation in Predicting Progression of Untreated High-grade Cervical Intraepithelial Neoplasia. *Clin Infect Dis* 2020;70:2582-90. doi:10.1093/cid/ciz677
26 Sand FL, Frederiksen K, Munk C, Jensen SM, Kjaer SK. Long-term risk of cervical cancer following conization of cervical intraepithelial neoplasia grade 3-A Danish nationwide cohort study. *Int J Cancer* 2018;142:1759-66. doi:10.1002/ijc.31202
27 Strander B, Andersson-Ellström A, Milsom I, Sparén P. Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study. *BMJ* 2007;335:1077-80. doi:10.1136/bmj.39363.471806.BE
28 Eurostat. Fertility Statistics, 2023. https://ec.europa.eu/eurostat/statistics-explained/SEPDF/cache/1273.pdf.
29 Wilkinson TM, Sykes PHH, Simcock B, Petrich S. Recurrence of high-grade cervical abnormalities following conservative management of cervical intraepithelial neoplasia grade 2. *Am J Obstet Gynecol* 2015;212:769.e1-7. doi:10.1016/j.ajog.2015.01.010
30 Hammer A, Demarco M, Campos N, et al. A study of the risks of CIN3+ detection after multiple rounds of HPV testing: Results of the 15-year cervical cancer screening experience at Kaiser Permanente Northern California. *Int J Cancer* 2020;147:1612-20. doi:10.1002/ijc.32950
31 Klügel S, Lücke C, Mehren A, et al. Patients with cervical intraepithelial neoplasms show different states of health-related quality of life and different coping styles depending on the choice of therapy: findings from the CIN study. *Int J Womens Health* 2019;11:511-7. doi:10.2147/IJWH.S208257
32 Carreon JD, Sherman ME, Guillén D, et al. CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. *Int J Gynecol Pathol* 2007;26:441-6. doi:10.1097/pgp.0b013e31805152ab
33 Damgaard RK, Jenkins D, Stoler MH, et al. High prevalence of HPV16 and high-grade cytology in women undergoing active surveillance for cervical intraepithelial neoplasia grade 2. *Acta Obstet Gynecol Scand* 2023;102:1227-35. doi:10.1111/aogs.14627

34 Chen Q, Du H, Pretorius RG, et al. High-Grade Cervical Intraepithelial Neoplasia Detected by Colposcopy-Directed or Random Biopsy Relative to Age, Cytology, Human Papillomavirus 16, and Lesion Size. *J Low Genit Tract Dis* 2016;20:207-12. doi:10.1097/LGT.0000000000000184
35 Kristensson JH, Sander BB, von Euler-Chelpin M, Lynge E. Predictors of non-participation in cervical screening in Denmark. *Cancer Epidemiol* 2014;38:174-80. doi:10.1016/j.canep.2013.12.007
36 Broberg G, Wang J, Östberg AL, et al. Socio-economic and demographic determinants affecting participation in the Swedish cervical screening program: A population-based case-control study. *PLoS One* 2018;13:e0190171. doi:10.1371/journal.pone.0190171
37 Arbyn M, Redman CWE, Verdoodt F, et al. Incomplete excision of cervical precancer as a predictor of treatment failure: a systematic review and meta-analysis. *Lancet Oncol* 2017;18:1665-79. doi:10.1016/S1470-2045(17)30700-3
38 Wentzensen N, Walker JL, Gold MA, et al. Multiple biopsies and detection of cervical cancer precursors at colposcopy. *J Clin Oncol* 2015;33:83-9. doi:10.1200/JCO.2014.55.9948

Web appendix: Supplementary tables and figures