Risk of cervical intraepithelial neoplasia grade 2 or worse in women aged ≥ 69 referred to colposcopy due to an HPV-positive screening test

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Abstract

Background Cervical cancer incidence and mortality rates are high in older women in many developed countries, including Denmark. Therefore, Danish women aged 69 and older were invited for one additional human papilloma virus (HPV) based screening test in 2017. Here, we describe the clinical management and detection rate of cervical intraepithelial neoplasia grade 2 or worse (CIN2 +) in screen-positive women referred for colposcopy.

Methods We conducted an observational study in public gynecology departments in Central Denmark Region, Denmark. Women were eligible for enrolment if they were aged 69 + in 2017, HPV positive on a screening test taken between April 20th, 2017, and December 31st, 2017, and had been referred for direct colposcopy. Data on participants' characteristics, colposcopic findings, and histological outcomes were collected from medical records and the Danish Pathology Databank. We estimated the proportion of women with CIN2 + at the first colposcopy visit and at end of follow up including 95% confidence intervals (CIs).

Results A total of 191 women were included with a median age of 74 years (IQR: 71—78). Most women (74.9%) did not have a fully visible transformation zone at colposcopy. At the first visit 170 women (89.0%) had a histological sample collected, 34 of whom (20.0%, 95% CI 14.3–26.8%) had CIN2 + diagnosed, 19 had CIN3 +, and two had cervical cancer). During follow-up additional CIN2 + were detected resulting in a total of 42 women (24.4%, 95% CI: 18.2–31.5%) being diagnosed with CIN2 +, 25 with CIN3 +, and three with cervical cancer. When restricting to women with paired histologic results (i.e., biopsies and a loop electrosurgical excision procedure (LEEP) specimen), we found that CIN2 + was missed in 17.9% (95% CI 8.9–30.4%) of biopsies compared to the LEEP.

Conclusion Our findings suggest a potential risk of underdiagnosis in older postmenopausal women referred to colposcopy**.** Future studies should explore potential risk-markers for discrimination of women at increased risk of CIN2 + from those at low risk, as this would reduce risk of underdiagnosis and overtreatment.

Keywords Cervical cancer screening, Human papillomavirus, Colposcopy, Cervical intraepithelial neoplasia, Loop electrosurgical excision procedure, Postmenopausal women

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Introduction

Cervical cancer incidence rates are higher in older women compared to younger women, and older women are more likely to be diagnosed with late-stage cervical cancer and die from the disease [1, 2]. Previous studies suggest that this may be because older women may not have had the opportunity to be screened and that cytology-based screening may fail to detect precancer and cancer in older women due to low sensitivity [3, 4]. These findings may suggest a need to continue screening beyond the age of 65 using a different screening method such as HPV-based screening. Several studies have clearly demonstrated that HPV-based screening is superior to cytology-based screening, also in older women [5–7]. However, diagnostic work-up of older women who screen positive is challenging, particularly because of atrophy and the retraction of the transformation zone, which makes complete visualization of the transformation zone (TZ) and potential lesions impossible. This may result in an increased risk of missing disease by up to twofold [8], potentially resulting in a delay in diagnosis [9]. To improve the detection and reduce risk of missing disease in this group of women, endocervical curettage (ECC) can be performed but remains controversial [10]. Although ECC may improve the detection rate of cervical precancer [11], not much is known about the diagnostic accuracy of ECC in older postmenopausal women. To ensure accurate diagnostics and treatment in women with incomplete visualization of the TZ some guidelines recommend that a diagnostic loop electrosurgical excision procedure (LEEP) be considered [12–14]. However, these recommendations are based on expert opinion as there is limited empirical evidence available on how to clinically manage older women.

In Denmark, women aged 69+(i.e., women born before 1948) were invited for an additional HPV-based screening test in 2017 [15] as an initiative to improve cervical cancer prevention among older birth cohorts with insufficient screening history [4]. Here, we aimed to describe clinical management and the proportion of women with CIN grade 2 or worse (CIN2+) in women aged 69+referred to colposcopy.

Materials and methods

We conducted an observational study from January to December 2020 in Central Denmark Region, which compromises approximately 20% of the Danish population. In Denmark, cervical cancer screening, including follow-up, diagnostic work-up and treatment, is free of charge. Women aged 23–64 are invited for regular screening every three to five years depending on age and screening method. From April 17 through December 31, 2017, women born before 1948 (i.e., aged 69+at that time)

were invited for one additional HPV screening test [4, 15]. HPV DNA testing was performed using the clinically validated COBAS 4800 assay (Roche Diagnostics), which allows for individual detection of HPV16 and HPV18, and pooled detection of 12 other high-risk HPV types (31,33,35,39,45,51,52,55,56,58,66,68). Women were referred directly for colposcopy if they were positive for HPV16 and/or HPV18, or if they were positive for other HPV types than HPV16/18 and had atypical squamous cells of undetermined significance or worse (ASC-US+) on reflex cytology. Women positive for HPV types other than HPV16/18 with normal cytology were recommended repeat HPV testing after one year and were only referred for colposcopy if HPV positive. Cytology was classified according to the 2014 Bethesda classification. As reported elsewhere, a total of 75,937 women were invited for screening in Central Denmark Region, 31.2% of whom participated, and the HPV prevalence was 4.8% in this population [16, 17] (Additional file 1).

Diagnostic work-up including colposcopies were performed in private gynecology clinics or public hospitals. During the study period there was no national clinical guidelines on how to clinically manage and treat HPV-positive women outside the typical screening age (i.e., age 65 and above). National clinical guidelines recommend that women of screening age should have biopsies collected regardless of the presence of visible lesions, with surgical treatment being recommended in the case of CIN2+in women outside of reproductive age [18]. Of note, collection of endocervical cytology or ECC is not routinely recommended in the Danish guidelines but may be performed depending on the colposcopist's preference. Of note, in this paper, ECC was mainly performed at one gynecological department.

Using electronic medical records, we identified women who were referred for colposcopy to a gynecology department at any public hospital in Central Denmark Region (i.e., Herning, Randers, Viborg, and Horsens) due to an HPV-positive screening test in the study period from April 20th 2017 to December 31st 2017. Eligible women had 1) a liquid-based cytology sample collected for HPV testing as part of the national one-time screening offer in the study period [15], and 2) were born before 1948. Women were excluded if their cervical sample was collected as part of follow-up for previous dysplasia, had undergone hysterectomy, or if they were examined in a private gynecology clinic as we had no access to medical records on these women.

From electronic medical records we collected information on colposcopic findings at the first visit including type of transformation zone (i.e., TZ1, TZ2, TZ3 as defined according to the 2011 International Federation of Cervical Pathology and Colposcopy nomenclature [19]).

Information on previous screening history (i.e., at least one record of screening vs no record of screening), and results of subsequent cervical biopsies, cervical smears, ECC, and LEEP were collected from the national Danish Pathology Databank [20]. The Danish Pathology Databank is a complete database that includes all pathology examinations dating back to 1997, and for some examinations back to 1970 [20]. Data was collected at an individual level using the central personal registration number (CPR), which is a unique code assigned to residents at birth or upon immigration [21]. Women were followed-up until December 31, 2020.

Histological diagnoses were graded according to the CIN classification system [22]; normal (including inflammation and non-specific reactive features), ungradable CIN (i.e., the full height of the epithelium is not discernible), CIN1, CIN2, CIN3, AIS, or cancer. If more than one histological diagnosis was recorded for the woman in the time span between the date of the HPV-positive screening test and end of follow-up (December 2020), the most severe diagnosis was used. Histological diagnoses were grouped into ≤ CIN1 (normal and CIN1) and CIN2 + (CIN2 and above), including ungradable CIN as these cases are clinically managed as CIN2 +. In case multiple HPV types were detected, we applied a hierarchical classification, assuming HPV 16 and/or 18 were the causal genotypes [23]. If both colposcopy-directed biopsies and random biopsies were taken, these cases were recorded as having directed biopsies taken. If it was not specified whether biopsies were directed or random, biopsies were recorded as random.

Our primary outcome was CIN2 + as this is the threshold for treatment in older women. Secondary outcomes were CIN3 + and cancer. We reported the overall number and proportion of CIN2 +, CIN3 +, and cancer after the first visit and at the end of follow-up, including corresponding 95% confidence intervals (95% CI). In a sub-analysis, we compared the CIN2 + detection in biopsies/ECC vs LEEP in women with paired histologic results. Data was entered and stored in REDCap [24]. Stata 16.1 analytic software (Stata Corp LP, College Station, TX) was used for data analysis.

Results

We identified 230 women born before 1948 who had a screening sample collected in the study period. Of these, 191 women (83.0%) met the inclusion criteria and were included (Fig. 1). The median age of included women was 74 years (IQR: 71—78) (Table 1). HPV 16 was the most common genotype (55.0%), followed by other high-risk types than HPV16/18 (29.8%), and HPV18 (15.2%) (Table 1). Most women (83.8%) had HPV 16, 18, or other HPV types only, while 16.2% had a mixture of HPV 16, 18 and/or other HPV types detected (data not shown). Most women (98.4%) had at least one record of a previous cervical cytology sample in the Danish Pathology Databank prior to 2017, 18.9% (*n* = 36) had a previous record of ASCUS + and/or CIN1 +, and 6.8% (*n* = 13) had a previous record of a LEEP (Table 1).

Among 191 HPV-positive women referred to colposcopy, most women had a TZ3 (*n* = 143, 74.9%) at the first colposcopy visit (Table 2). In 171 (89.5%) of these
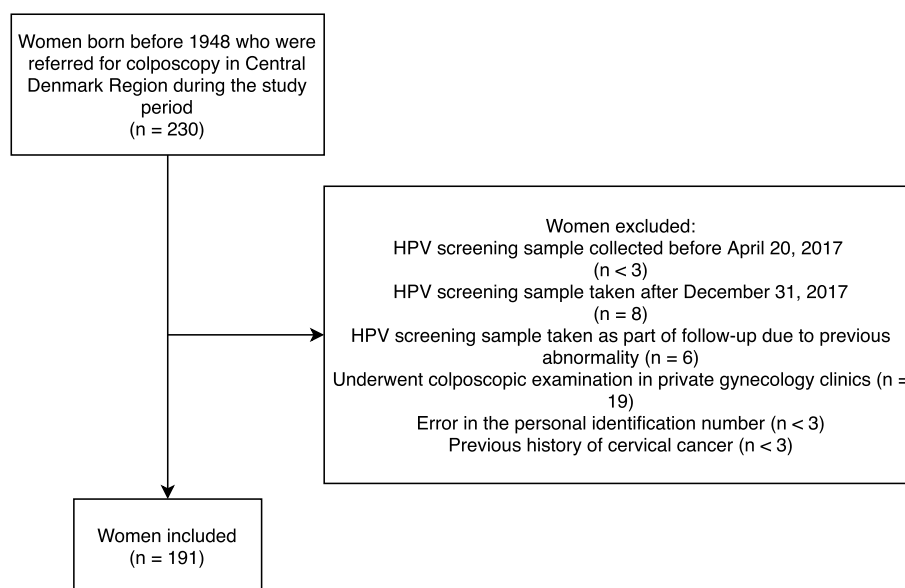
Fig. 1 Flow diagram of women included in the present study

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Table 1 Basic characteristics of women born before 1948 who were referred for colposcopy due to an HPV-positive screening test (*n* = 191)

	n (%)
Median age in years (IQR)	74 (71–78)
HPV type in referral screening test[a]	
HPV 16	105 (55.0%)
HPV 18	29 (15.2%)
Other high-risk HPV	57 (29.8%)
Cytology triage in baseline referral screening test in women with other high-risk HPV	
Normal	6 (10.5%)
ASCUS	13 (22.8%)
LSIL	11 (19.3%)
ASC-H	12 (21.1%)
HSIL	15 (26.3%)
Number of colposcopy visits	
1	134 (70.2%)
2	36 (18.8%)
3	16 (8.4%)
4	5 (2.6%)
At least one previous record of a cervical smear[b]	
Yes	188 (98.4%)
No	3 (1.6%)
Previous record of ASCUS + or CIN1 +[b]	
Yes	36 (18.9%)
No	155 (81.2%)
Previous record of a LEEP[b]	
Yes	13 (6.8%)
No	178 (93.2%)

Of note, percentages may add up to more than 100% due to rounding

Abbreviations: IQR Inter quartile range, *ASCUS* Atypical Squamous Cells of Undetermined Significance, *LSIL* Low-grade Squamous Intraepithelial Lesions, *ASC-H* Atypical squamous cells, cannot exclude High-grade, *HSIL* High grade squamous intraepithelial lesion, *LEEP* Loop Electrosurgical Excision Procedure

[a] Hierarchical classification of HPV genotypes

[b] Previous record in the Danish Pathology Databank

women no colposcopic abnormalities were reported (Table 2), and most women had random biopsies (75.9%) collected. Reasons for abstaining from collection of biopsies at the first visit were mainly cervical stenosis, no visible lesions, or patient wishes (data not shown). A total of 170 women (89.0%) had histological samples collected (i.e., biopsies, ECC, or LEEP) at or immediately after the first colposcopy visit, with most women having biopsies collected (94.7%, 161/170). In these biopsies the TZ was not represented in 65.8% (106/161) of women, while 32.9% (53/161) of women had the TZ represented in at least one biopsy, and in 1.2% (2/161) of women it was not reported. Of women who had a histological sample collected, 34 (20.0%,

95% CI 14.3–26.8%) women had CIN2 + diagnosed, 19 (11.2%, 95% CI 6.9 – 16.9) had CIN3 +, and two (1.2%, 95% CI 0.1 – 4.2%) were diagnosed with cervical cancer (Table 2).

Compared to women diagnosed with CIN1 at the first visit, women diagnosed with CIN2 + were significantly more likely to have a TZ1 (23.5% vs 5.2%), have any abnormality detected (20.6% vs 9.6%), and have undergone a diagnostic LEEP (20.6% vs. 7.3%) (Table 2). We found no difference between the two groups with respect to ECC.

With respect to data from the entire study period, a total of 172 (90.1%) had one or more histological samples collected (i.e., biopsy, ECC or LEEP). Of these, 42 women (24.4%, 95% CI: 18.2–31.5%) had CIN2 + detected, 25 (14.5%, 95% CI 9.6 – 20.7) had CIN3 + detected, and three (1.7%, 95% CI 0.4 – 5.0) were diagnosed with cervical cancer.

LEEP was performed in 62 women (32.4%) at some point during the study, with 56 (29.3%) having both cervical biopsies and/or ECC and a LEEP performed. Despite having no evidence of CIN2 + in the biopsies or the ECC, 10 women had CIN2 + (10/56, 17.9%, 95% CI 8.9–30.4%) detected in the LEEP specimen (data not shown). When restricting to women with biopsies and LEEP, similar findings were found.

Discussion

In this group of older HPV-positive women referred for colposcopy, 20.0% had CIN2 + detected at the first visit and 24.4% had CIN2 + detected at some point during the study period. Most women diagnosed with CIN2 + had no abnormalities visualized at colposcopy. Cervical biopsies/ECC underestimated the detection of CIN2 + by 17.9% compared with the LEEP result. As 73.3% of women only had cervical biopsies or an ECC collected during the study period, despite having a TZ3 at colposcopy, the proportion of CIN2 + reported in this study is likely underestimated. Further, the majority of biopsies taken (65.8%) did not include the TZ, likely contributing further to the underestimation of high-grade disease.

The proportion of women with CIN2 + in our study (20.0% and 24.4%) was comparable to that reported in a previous Swedish study (23%) on older women with a persistent HPV infection but was higher than the one reported in a previous population-based Danish study (18%) [17, 25, 26]. Given differences in screening and referral strategy, age of the population, proportion of women with a TZ3, and clinical management, it is challenging to make any formal comparison across studies.

According to a Swedish study collection of random biopsies and ECC were useful clinical tools in detecting CIN2 + in older women with TZ3 in the absence of

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Table 2 Colposcopic findings at the first colposcopy visit among HPV screening-positive women, overall and stratified by histology

| | All women (n = 191, 100%) | | Histological sample collected[c] (n = 170) (%) | | | |
| | | | ≤ CIN1 (n = 136, 80%) | | CIN2 + (n = 34, 20%) | |
	n (%)	(95% CI)	n (%)	(95% CI)	n (%)	(95% CI)
Transformation zone type recorded						
TZ1	15 (7.9%)	4.5–12.6%	7 (5.2%)	2.1–10.3%	8 (23.5%)	10.8–41.2%
TZ2	16 (8.4%)	4.9–13.2%	15 (11.0%)	6.3–17.5%	< 3	-
TZ3	143 (74.9%)	68.1–80.9%	101 (74.3%)	66.1–81.4%	22 (64.7%)	46.5–80.3%
Not reported	17 (8.9%)	5.3–13.9%	13 (9.6%)	5.2–15.8%	3 (8.8%)	1.9–23.7%
Colposcopic abnormality[a]						
Acetowhite	10 (5.2%)	2.5–9.4%	6 (4.4%)	1.6–9.4%	4 (11.8%)	3.3–27.5%
Punctuations or mosaic vessel patterns	5 (2.6%)	0.9–6.0%	2 (1.5%)	0.2–5.2%	3 (8.8%)	1.9–23.7%
Atypical vessels	11 (5.6%)	2.9–10.1%	8 (5.9%)	2.6–11.3%	3 (8.8%)	1.9–23.7%
Discoloration	< 3		< 3		0	
No visible lesions	171 (89.5%)	84.3–93.5%	123 (90.4%)	84.2–94.8%	27 (79.4%)	62.1–91.3%
Median number of biopsies collected (range)	3 (0–7)		3 (1–7)		4 (1–7)	
Type of histological sample[b]						
Colposcopy-directed biopsies	17 (8.9%)	5.3–13.9%	12 (8.8%)	4.6–14-9%	5 (14.7%)	5.0–31.1%
Random biopsies	145 (75.9%)	69.2–81-8%	116 (84.6%)	78.2–90.8%	28 (82.4%)	65.5–93.2%
Endocervical curettage	29 (15.2%)	10.4–21.1%	23 (16.9%)	11.0–24.3%	5 (14.7%)	5.0–31.1%
Diagnostic LEEP	17 (8.9%)	5.3–13.9%	10 (7.3%)	3.5–13.1%	7 (20.6%)	8.7–37.9%
LEEP due to abnormal histological sample	22 (11.5%)	7.3–16.9%	0	-	22 (64.7%)	46.5–80.3%

Of note, percentages may add up to more than 100% due to rounding

[a] Adds to more than 100% as some women had more than one abnormality detected at colposcopy

[b] If both colposcopy-directed biopsies and random biopsies were taken, these cases were recorded as having directed biopsies taken. If it was not specified whether biopsies were directed or random, these cases were recorded as random

[c] Based on worst histopathological examination of cervical biopsies, ECC and/or LEEP

visible lesions [25]. Our results, however, indicate that we should be cautious when relying only on blind random biopsies, even when a median of 3 biopsies were taken. We found that biopsies underestimated CIN2 + in 17.9% of cases compared with the LEEP diagnosis, which is similar to Gustafson et al. who showed a 17.7% difference in older women who all underwent both biopsy and LEEP [8]. These findings suggest that women with TZ3 are at increased risk of having disease missed and that collection of blind biopsies may result in an insufficient diagnostic work-up because the lesion may be located high in the cervical canal. Therefore, colposcopists should be cautious when relying solely on the result of cervical biopsies in this group of women as a negative biopsy result does not necessarily infer no cervical disease is present. Given that national estimations of CIN2 + and CIN3 + detection rates in older women may be based on the results of cervical biopsies, immediate risk-assessment may be biased as well.

According to the British, Swedish, Australian, and American guidelines, a diagnostic LEEP may be considered when the squamocolumnar junction is not fully visualized [12–14, 27]. However, performing a LEEP in all HPV-positive older women will likely lead to overtreatment and potential surgical complications, such as stenosis, which may compromise subsequent follow-up. HPV may also still be present after a LEEP as it has been shown that HPV clearance may be delayed after LEEP, especially in older women [28]. Balancing the harms of potential overtreatment versus underdiagnosis is of great importance in clinical management of older HPV-screen-positive women referred for colposcopy. Referral for colposcopy is a known harm of screening as it can lead to psychological distress and anxiety [29–31]. However, a previous study has found lower anxiety levels in older women undergoing colposcopy and treatment (median age 67.7 years) compared to women aged 23–50 [32, 33]. Additionally, a recent Danish qualitative study demonstrated that older post-menopausal women preferred diagnostic LEEP over continued surveillance, even if this would result in increased risk of overtreatment [34]. From a clinical perspective, a shared decision-making tool could be helpful for women and their health-care provider as this may enable a better discussion of pros

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and cons, particularly because the current literature on this subject is limited.

The subject of when women should exit the cervical cancer screening program has gained much attention lately [35], particularly due to decreasing hysterectomy rates and an increasing life expectancy, leaving more women at-risk. However, as the preventive effect of cervical cancer screening not only relies on the uptake and effectiveness of the screening test but also on accurate diagnostics of those who screen positive, it is critical that clinicians ensure accurate diagnostics and adequate treatment of screen-positive women with minimal harm if screening is to be performed in this population. In our study it was clear that women were followed differently depending on hospital traditions and colposcopists' experience. For example, ECC was only performed in 16.8% of women, and these were mainly performed in one hospital.

Further research is needed to correctly identify the HPV-positive older women who are at greatest risk of CIN2+. Cytology triage may not be the best solution in older HPV-positive women as cytology has been shown to have a lower sensitivity in this population [36]. Furthermore, triage by partial HPV genotyping (i.e., HPV 16/18) may also be suboptimal as the relative contribution of HPV16/18 in cervical cancer declines with increasing age, reaching about 45% in women aged 70+. [23] Biomarkers like p16/Ki67 dual stain-cytology or DNA methylation of certain host genes show promising results in triage of younger women who screen HPV positive [37, 38]. Nevertheless, these results may not be generalizable to an older population of women, where collection of cervical samples is challenging. However, a recent Danish study on screen-positive postmenopausal women suggested that p16/Ki67 dual stain-cytology can be a useful tool to determine which women can safely undergo repeated cervical sampling rather than a diagnostic LEEP [39]. This could potentially minimize the risk of overtreatment while reducing risk of underdiagnosis. Before better triage methods are widely available, clear consensus guidelines on how to best manage and follow-up these older women are critically needed.

Strengths and limitations
Strengths of this study were the use of individual-level data from electronic medical records. Further, we used the national Danish Pathology Databank, which ensured a complete record of previous cervical cancer screening history and valid results on histopathologic outcomes at an individual level. Limitations of the study were that those women with HPV16 or 18 were referred directly for colposcopy and women with non-HPV16/18 genotypes were only referred directly for colposcopy if cytology was abnormal (ASC-US+). Therefore, we were unable to include women with non-HPV 16/18 and normal cytology. With this selection bias it appears that HPV16 was the most common HPV type in these women despite that a previous study reported non-HPV16/18 genotypes to be more common in older women compared to HPV16 and 18 [40]. This may have underestimated the true prevalence of non-HPV 16/18, and may have affected the generalizability of our data to other populations. One could anticipate that this selection could also have underestimated the detection rate of CIN2+. However, this limitation is considered less likely as this research group previously found that older women found to be HPV positive were more likely to have CIN2+ present in LEEP if cytology was abnormal than if cytology had been normal at referral [8]. Due to the lack of clinical guidelines, women were managed differently between the hospitals and within each hospital dependent on the colposcopist. This makes it difficult to compare data but reflects the everyday clinical challenges colposcopists are facing. Finally, the small sample size makes the results less robust.

Conclusion
We found that one in four women aged 69+ referred for colposcopy due to an HPV-positive screening test were diagnosed with CIN2+. However, the overall proportion of CIN2+ may be underestimated as 73.3% of women with TZ3 had insufficient diagnostic work-up. Future studies should explore the use of biomarkers for identification of screen-positive postmenopausal women who at the first colposcopy visit could be at increased risk for CIN2+, thereby reducing risk of overtreatment and simultaneously secure adequate diagnostics. In the meantime, shared decision making between the clinician and woman discussing the pros and cons of repeated cervical follow-up versus diagnostic LEEP will be essential.

Abbreviations

95% CI	95% Confidence interval
CIN	Cervical Intraepithelial Neoplasia
ECC	Endocervical Curettage
HPV	Human papillomavirus
IQR	Interquartile range
LEEP	Loop Electrosurgical Excision Procedure
TZ	Transformation Zone

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12885-023-10888-1.

Additional file 1. Flow diagram of Women aged 69+ (i.e., women born before 1948) invited for an additional HPV-based screening test in Central Denmark Region.

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Availability of data and materials
The data that support the findings of this study are available from each hospital administration, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of each hospital administration.

Declarations

Ethics approval and consent to participate
As the study was classified as a quality assurance study according to Danish guidelines, the study was registered and approved on January 28, 2020 by the hospital administration in each of the four participating hospitals, as required by Danish legislation (no record number available). Only medical records of patients were used and the informed consent was waived by approval of each of the hospitals administrations in the participating hospitals. The approving hospitals are Randers Regional Hospital, Horsens Regional Hospital, Viborg Regional Hospital and Goedstrup Hospital. All methods were therefore carried out in accordance with relevant guidelines and regulations in Denmark.

Consent for publication
Not applicable.

Competing interests
AH, LWG, and MT have received a speaker's fee from Astra Zeneca and reagents for free from Roche, Denmark, outside the submitted work. The remaining authors declare no conflicts of interest.

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References
1. Hammer A, Kahlert J, Gravitt PE, Rositch AF. Hysterectomy-corrected cervical cancer mortality rates in Denmark during 2002–2015: a registry-based cohort study. Acta Obstet Gynecol Scand. 2019;98(8):1063–9.
2. Hammer A, Kahlert J, Rositch A, Pedersen L, Gravitt P, Blaakaer J, Soegaard M. The temporal and age-dependent patterns of hysterectomy-corrected cervical cancer incidence rates in Denmark: a population-based cohort study. Acta Obstet Gynecol Scand. 2017;96(2):150–7.
3. Hammer A, Soegaard V, Maimburg RD, Blaakaer J. Cervical cancer screening history prior to a diagnosis of cervical cancer in Danish women aged 60 years and older—A national cohort study. Cancer Medi. 2019;8(1):418–27.
4. Hammer A, Hee L, Blaakær J, Gravitt P. Temporal patterns of cervical cancer screening among Danish women 55 years and older diagnosed with cervical cancer. J Low Genit Tract Dis. 2018;22(1):1–7.
5. Cuzick J, Clavel C, Petry KU, Meijer CJLM, Hoyer H, Ratnam S, Szarewski A, Birembaut P, Kulasingam S, Sasieni P, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. Int J Cancer. 2006;119(5):1095–101.
6. Ronco G, Dillner J, Elfström KM, Tunesi S, Snijders PJF, Arbyn M, Kitchener H, Segnan N, Gilham C, Giorgi-Rossi P, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383(9916):524–32.
7. Castle PED, Stoler MHP, Wright TCP, Sharma AP, Wright TLMD, Behrens CMMD. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol. 2011;12(9):880–90.
8. Gustafson LW, Hammer A, Bennetsen MH, Kristensen CB, Majeed H, Petersen LK, et al. Cervical intraepithelial neoplasia in women with transformation zone type 3: cervical biopsy versus large loop excision. BJOG: an international journal of obstetrics and gynaecology. 2022;129(13):2132–40.
9. Gustafson LW, Petersen LK, Bor P, Andersen B, Hammer A. Cervical cancer prevention among older women - challenges in screening, diagnostic workup, and treatment. Acta Obstet Gynecol Scand. 2021;100(8):1364–8.
10. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, Huh WK, Kim JJ, Moscicki A-B, Nayar R, et al. 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. J Low Genit Tract Dis. 2020;24(2):102–31.
11. Pretorius RG, Zhang WH, Belinson JL, Huang MN, Wu LY, Zhang X, Qiao YL. Colposcopically directed biopsy, random cervical biopsy, and endocervical curettage in the diagnosis of cervical intraepithelial neoplasia II or worse. Am J Obstet Gynecol. 2004;191(2):430–4.
12. Guidelines for the management of screen-detected abnormalities, screening in specific populations and investigation of abnormal vaginal bleeding. Available from: https://wiki.cancer.org.au/australia/Guidelines:Cervical_cancer/Screening.
13. SFOG-riktlinje av NVP för cervixcancerprevention. https://www.sfog.se/media/336482/sfog-riktlinje-190330_revhs-190413.pdf.
14. NHS, Cervical screening: programme and colposcopy management. https://www.gov.uk/government/publications/cervical-screening-programme-and-colposcopy-management.
15. Danish Government DR, Danish Municipalities. Action plan for cancer plan IV (2017–2020). 2017.
16. DKLS. Resultater fra Engangstilbuddet: Livmoderhalskræftscreening blandt danske kvinder født før 1948 1. delrapport. 2019.
17. St-Martin G, Viborg PH, Andersen ABT, Andersen B, Christensen J, Ejersbo D, Heje HN, Jochumsen KM, Johansen T, Larsen LG, et al. Histological outcomes in HPV-screened elderly women in Denmark. PLoS One. 2021;16(2):e0246902–e0246902.
18. Danish Society of Obstetrics and Gynaecology Guideline. Udredning, behandling og kontrol af cervikal dysplasi (in Danish only). 2019. http://gynobsguideline.dk/hindsgavl/Cervixdysplasi2012.pdf.
19. Quaas J, Reich O, Küppers V. Explanation and use of the Rio 2011 colposcopy nomenclature of the IFCPC (International Federation for Cervical Pathology and Colposcopy): comments on the general colposcopic assessment of the uterine cervix: adequate/inadequate; squamocolumnar junction; transformation zone. Geburtshilfe und Frauenheilkunde. 2014;74(12):1090–2. https://doi.org/10.1055/s-0034-1383216.
20. Erichsen R, Lash TL, Hamilton-Dutoit SJ, Bjerregaard B, Vyberg M, Pedersen L. Existing data sources for clinical epidemiology: the Danish National Pathology Registry and Data Bank. Clin Epidemiol. 2010;2:51–6.
21. Pedersen CB. The Danish Civil Registration System. Scand J Public Health. 2011;39(7_suppl):22–5.
22. Richart RM. Natural history of cervical intraepithelial neoplasia. Clin Obstet Gynecol. 1967;10(4):748–84.

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23. Hammer A, Rositch A, Qeadan F, Gravitt PE, Blaakaer J. Age-specific prevalence of HPV16/18 genotypes in cervical cancer: a systematic review and meta-analysis. Int J Cancer. 2016;138(12):2795–803.
24. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, McLeod L, Delacqua G, Delacqua F, Kirby J, et al. The REDCap consortium: building an international community of software platform partners. J Biomed Inform. 2019;95:103208–103208.
25. Sahlgren H, Elfström KM, Lamin H, Carlsten-Thor A, Eklund C, Dillner J, Elfgren K. Colposcopic and histopathologic evaluation of women with HPV persistence exiting an organized screening program. Am J Obstet Gynecol. 2020;222(3):253.e251-253.e258.
26. Bergengren L, Karlsson MG, Helenius G. Prevalence of HPV and pathological changes among women 70 years of age, 10 years after exclusion from the Swedish cervical cancer screening program. Cancer Causes Control. 2020;31(4):377–81.
27. Cheung LC, Egemen D, Chen X, Katki HA, Demarco M, Wiser AL, Perkins RB, Guido RS, Wentzensen N, Schiffman M. 2019 ASCCP risk-based management consensus guidelines: methods for risk estimation, recommended management, and validation. J Low Genit Tract Dis. 2020;24(2):90–101.
28. Bergengren L, Lillsunde-Larsson G, Helenius G, Karlsson MG. HPV-based screening for cervical cancer among women 55–59 years of age. PLoS One. 2019;14(6):e0217108–e0217108.
29. O'Connor M, Gallagher P, Waller J, Martin CM, O'Leary JJ, Sharp L. Adverse psychological outcomes following colposcopy and related procedures: a systematic review. BJOG. 2016;123(1):24–38.
30. Forss A, Tishelman C, Widmark C, Sachs L. Women's experiences of cervical cellular changes: an unintentional transition from health to liminality? Sociol Health Illn. 2004;26(3):306–25.
31. Swancutt DR, Greenfield SM, Luesley DM, Wilson S. Women's experience of colposcopy: a qualitative investigation. BMC Womens Health. 2011;11(1):11–11.
32. Hellsten C, Sjostrom K, Lindqvist PG. A 2-year follow-up study of anxiety and depression in women referred for colposcopy after an abnormal cervical smear. BJOG. 2008;115(2):212–8.
33. Gustafson LW, Larsen MB, Hammer A, Petersen LK, Andersen B, Bor P. Levels of anxiety in women aged ≥45 years undergoing diagnostic large loop excision of the transformation zone: a longitudinal study. BJOG. 2023;130(2):192–200.
34. Kirkegaard P, Gustafson LW, Petersen LK, Andersen B. 'I want the whole package'. Elderly patients' preferences for follow-up after abnormal cervical test results: a qualitative study. Patient Prefer Adherence. 2020;14:1185–93.
35. Gravitt PE, Landy R, Schiffman M. How confident can we be in the current guidelines for exiting cervical screening? Prev Med. 2018;114:188–92.
36. Gyllensten U, Gustavsson I, Lindell M, Wilander E. Primary high-risk HPV screening for cervical cancer in post-menopausal women. Gynecol Oncol. 2012;125(2):343–5.
37. Sahasrabuddhe VV, Luhn P, Wentzensen N. Human papillomavirus and cervical cancer: biomarkers for improved prevention efforts. Future Microbiol. 2011;6(9):1083–98.
38. Clarke MA, Cheung LC, Castle PE, Schiffman M, Tokugawa D, Poitras N, Lorey T, Kinney W, Wentzensen N. Five-year risk of cervical precancer following p16/Ki-67 dual-stain triage of HPV-positive women. JAMA Oncol. 2018;5(2):181–6.
39. Gustafson LW, Tranberg M, Christensen PN, Brøndum R, Wentzensen N, Clarke MA, et al. Clinical utility of p16/Ki67 dual stain cytology for detection of cervical intraepithelial neoplasia grade two or worse in women with a transformation zone type 3: a cross sectional study. BJOG. 2023;130(2):202–9.
40. Andersen B, Christensen BS, Christensen J, Ejersbo D, Heje HN, Jochumsen KM, Johansen T, Larsen LG, Lynge E, Serizawa R, et al. HPV-prevalence in elderly women in Denmark. Gynecol Oncol. 2019;154(1):118–23.

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