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# BMJ Open Performance of HPV E4 and p16<sup>INK4a</sup> biomarkers in predicting regression of cervical intraepithelial neoplasia grade 2 (CIN2): protocol for a historical cohort study

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## ABSTRACT

**Introduction** Cervical intraepithelial neoplasia grade 2 (CIN2) represents a spectrum of lesions with variable progression and regression. Pathological diagnosis of CIN2 is subjective and poorly reproducible. Accurate diagnosis and identification of different patterns of CIN2 related to outcome are essential to reduce the risks of overtreatment or undertreatment. It is important to explore novel methods for risk stratification of CIN2 to enable targeted treatment of women at high risk of progression or persistent disease and follow-up of women at low risk. The combination of the novel biomarker human papillomavirus (HPV) E4 with p16<sup>INK4a</sup> targets steps in the transition from a productive oncogenic HPV infection (CIN1) to a transformed lesion (CIN3) within CIN2. Previous cross-sectional studies suggest that HPV E4 combined with p16<sup>INK4a</sup> may be valuable for risk assessment of CIN2. However, data on HPV E4/p16<sup>INK4a</sup> as a predictor for CIN2 regression is lacking.

**Methods and analysis** We will conduct a historical cohort study including 500 women aged 23–40 years with a first CIN2 diagnosis in Aarhus, Denmark during 2000–2010. Women will be eligible if they have undergone active surveillance and have no previous record of hysterectomy, cone biopsy, and CIN2 or worse. Women will be randomly selected through the Danish Pathology Databank. Tissue samples from women included will be sectioned for p16<sup>INK4a</sup> and HPV E4 immunohistochemical staining in addition to conventional hematoxylin and eosin (H&E) staining. A positive result will be defined as HPV E4 positive. Through the Danish Pathology Databank, we will collect results on all subsequent cervical biopsies. Regression will be used as the primary outcome.

**Ethics and dissemination** The study has been approved by the Ethical Committee in Central Denmark Region (1-10-72-60-20) and registered at the Faculty of Health, Aarhus University. Results will be published in a peer-reviewed journal and presented at scientific meetings.

**Trial registration number** NCT05049252.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is the first study to examine combined human papillomavirus HPV E4 and p16<sup>INK4a</sup> immunohistochemistry as a predictor for cervical intraepithelial neoplasia grade 2 (CIN2) regression in a large cohort (N=500).
- ⇒ The study design (ie, cohort study) enables an estimation of risk of CIN2 evolution, which is highly important for clinical counselling.
- ⇒ Active surveillance of CIN2 has been common practice in the study setting for the past 25 years.
- ⇒ Review of slides by an international expert panel will improve the external validity.
- ⇒ Cervical punch biopsies will be used as the proxy measure for in vivo conditions.

## INTRODUCTION

Cervical cancer remains one of the leading cancers among women globally, accounting for 600 000 incidental cases and 300 000 deaths in 2020.<sup>1–3</sup> The disease can be prevented through prophylactic human papillomavirus (HPV) vaccination of women without HPV infection where it is available but currently, mostly through cervical cancer screening, which allows for detection and subsequent treatment of cervical precancer, thereby preventing progression to cancer.

Cervical precancer arising in the squamous epithelium is classified as cervical intraepithelial neoplasia (CIN), and is graded as CIN1, CIN2 or CIN3 depending primarily on the proportion of the squamous epithelial layer occupied by proliferating dysplastic cells.<sup>4 5</sup> CIN2 is a complex and equivocal diagnosis representing a mixture of both CIN1-like HPV-producing and CIN3-like transformed neoplastic lesions with different risks of progression.<sup>4 6 7</sup> The CIN2 diagnosis



is associated with low reproducibility and high risk of misclassification, potentially resulting in important risk of overtreatment and undertreatment.<sup>6–8</sup>

Historically, CIN2 has been the threshold for excisional treatment in most developed countries for at least 40 years.<sup>8</sup> However, due to high regression rates of CIN2 of around 50%, especially among women under the age of 30 around 60%, and increased risk of preterm birth after excisional treatment, several countries have switched to active surveillance of CIN2 in young women who wish to have children.<sup>9–12</sup> In Denmark, active surveillance (ie, cervical smear, colposcopy and cervical punch biopsies every 4–6 months depending on the histopathological result at each visit) has been recommended for such women since 2012, and in Central Denmark Region since 1995.<sup>13 14</sup> However, knowledge on risk markers predictive for CIN2 evolution during surveillance is limited. The long-term practice of active surveillance in the Central Denmark Region, with archived samples and follow-up data from nationwide registers, provides a unique opportunity to explore risk markers for CIN2 regression. One potential marker is the novel immunohistochemical biomarker *HPVE4*, which previous studies have shown to support reproducible diagnosis and stratification of CIN2 into CIN1-like (productive) and CIN3-like (transformed) phenotypes when used in combination with p16<sup>INK4a</sup>.<sup>15–18</sup> However, the performance of HPV E4/p16<sup>INK4a</sup> as a predictor for CIN2 regression requires further studies. We will explore whether the HPV E4 biomarker, in addition to p16<sup>INK4a</sup>, can be used for risk stratification of CIN2.

## METHODS AND ANALYSIS

### Cervical cancer screening and clinical follow-up in Denmark

Cervical cancer screening was launched as opportunistic screening in parts of Denmark during the 1960s, and the first national guidelines were published in 1986.<sup>19</sup> Initially, screening was recommended for women aged 23–59 every 3 years, but since 2007 Danish women aged 23–64 years have been invited for regular screening every three to 5 years depending on the age of the woman.<sup>19–21</sup> Of note, screening is currently transitioning from cytology to primary HPV screening for women aged 30 years and older. Screening, clinical follow-up and subsequent treatment are free of charge for all Danish citizens.<sup>20–22</sup>

In Denmark, women with an abnormal screening test may undergo repeat testing or be referred to colposcopy depending on the screening test result. At colposcopy, targeted biopsies are collected and if no lesion is detected, collection of multiple random biopsies is recommended.<sup>14</sup> Women with CIN1 are recommended repeat smear after 1 year while excisional treatment is recommended in women diagnosed with CIN3 or adenocarcinoma in situ (AIS).<sup>19</sup> In Central Denmark Region, women of reproductive age (ie, <40 years) diagnosed with CIN2 are recommended active surveillance if they wish to have children in the future. This includes a smear, colposcopy and cervical biopsies every 4–6 months for

up to 2 years, with excisional treatment being recommended in the case of progression to CIN3+ or persistent CIN2 after 2 years of follow-up. There are no additional specific requirements (ie, lesion size, cytology result, HPV status or compliance) for being eligible for active surveillance.<sup>14 19</sup>

### Danish Pathology Databank

The Danish Pathology Databank was established in 1997 and stores information on all cytopathological and histopathological examinations performed in Denmark since 1997. Although most pathology departments have transferred data prior to 1997, data are considered incomplete prior to this date. All samples registered in the Danish Pathology Databank are specified by the personal identification number (ie, a unique code assigned to all Danish residents at birth or on immigration), sample number, diagnosis code (SNOMED), procedure, method, sample type, department and the examining pathologist.<sup>23 24</sup> In Denmark, all histology samples are stored permanently in local pathology archives after the examination. Slides used for morphological assessment (cytology and histology) are stored in local archives for 10 years after which they are destroyed.

### SNOMED nomenclature

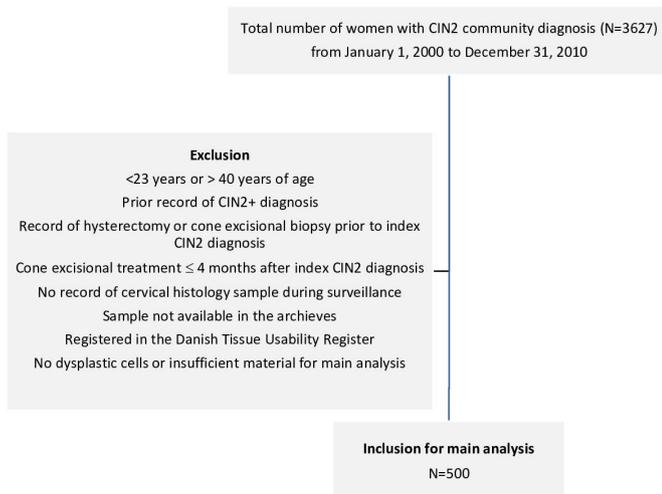
Since 1983 all pathology samples analysed in Denmark have been classified according to the international SNOMED) nomenclature and classification system (1993, 3. Edition). The SNOMED terminology is based on unique codes for functional and anatomical conditions, procedures and treatment specified as: morphology (M), topography (T), aetiology (Æ), function (F), disease (S), P (procedure) codes.<sup>23 25</sup> Prior to, 2011 all cervical histology samples have been classified morphologically using SNOMED codes for dysplasia and since then, the CIN nomenclature (WHO 2003) has been used.<sup>5 26</sup> In the present study, CIN2 refers to women diagnosed prior to treatment with moderate dysplasia or CIN2.

### Study design and population

We will conduct a historical cohort study. The source population will comprise all women with a record of a CIN2 diagnosis in the Danish Pathology Databank at the Department of Pathology, Aarhus University Hospital, Denmark from 1 January 2000 through 31 December 2010.

### Selection and eligibility

Women will be eligible for inclusion if they fulfil the following criteria: (1) age 23–40 at the time of first CIN2 diagnosis, (2) no record of excisional treatment of the cervix within 4 months after their index CIN2 diagnosis, indicating they underwent active surveillance, (3) have a subsequent record of at least one histopathological examination of a cervical punch biopsy during the active surveillance period of 2 years. Women will be excluded if they have a previous record of CIN2+ diagnosis, hysterectomy



**Figure 1** Selection flow chart. CIN2, cervical intraepithelial neoplasia grade 2; CIN2+, CIN2, CIN3 or worse cervical epithelial lesion.

or excisional treatment of the cervix recorded in the Danish Pathology Databank.

Women with a record of <CIN2 after incident CIN2 diagnosis will be defined as ‘regression’. This will be based on histopathological examination of a cone biopsy specimen or cervical punch biopsies. In contrast, women with a subsequent record of CIN2, CIN3 or cancer during the 2-year surveillance period, assessed either on cone biopsy specimen or cervical punch biopsies, will be classified as ‘non-regression’. The study will be based on the worst histopathological diagnosis registered during the surveillance period.

Eligible women will be identified through the Danish Pathology Databank at the Department of Pathology, Aarhus University Hospital using the SNOMED codes for moderate dysplasia (M74A09) and cervical punch biopsy (T83110). From the source population we will randomly select 500 women for analysis, equally distributed within each calendar year during the study period (figure 1). We will collect formalin-fixed and paraffin-embedded (FFPE) tissue blocks from local archives at the Department of Pathology, Aarhus University Hospital. From each tissue block, we will cut a section for hematoxylin and eosin (H&E) staining to evaluate the quality of the tissue (ie, sufficient material, tangential orientation, squamocolumnar junction represented, etc), and to ensure that dysplastic squamous epithelial cells are represented but there will be no additional evaluation of the lesion grade at this stage.

## Biomarkers and laboratory analysis

### Conventional p16<sup>INK4a</sup> biomarker

The p16 protein plays an important role as cyclin dependent kinase inhibitor in the retinoblastoma protein mediated control of cellular progression and differentiation.<sup>27 28</sup> In CIN, the p16 protein is over expressed in dysplastic cells compared with normally differentiated cells, and is used to indicate its important role in

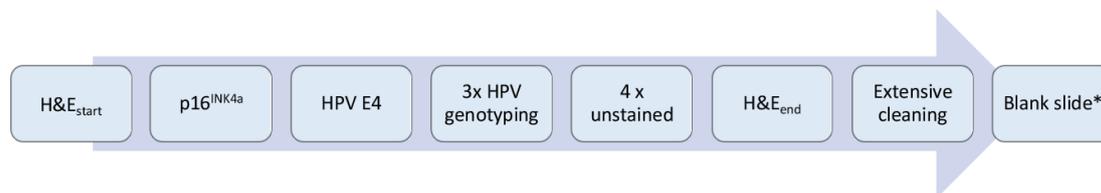
carcinogenic transformation of these cells.<sup>28</sup> Consequently, p16<sup>INK4a</sup> is used as a marker in the interpretation of cervical grade of lesion.<sup>29–31</sup> According to the US Lower Anogenital Squamous Terminology (LAST) for the diagnosis of high-grade CIN, diffuse expression of the p16<sup>INK4a</sup> biomarker is used to strengthen diagnosis and support H&E morphological analysis.<sup>4 32 33</sup>

### Novel HPV E4 biomarker

The E4 protein is functionally expressed by the E1 ^E4 mRNA gene product and plays a central role in viral genome amplification attainment and synthesis during HPV life cycle. In the initial replication phase, the E1 ^E4 gene product is undetectable in the host cells but often becomes detectable at the onset of viral genome amplification entering the late stage of productive infection.<sup>34–36</sup> Prior studies have shown that the E4 protein plays an important role in the incorporation of HPV DNA in the host cells, and the expression of the E4 protein inversely correlates with grade of CIN, that is, high and frequent expression in CIN1, with no or minimal expression in CIN3.<sup>18</sup> Thus, these studies suggest that the biomarker HPV E4 in combination with p16<sup>INK4a</sup> and morphology analysis might enable to reliably discriminate between CIN1-like (HPV E4 positive) productive lesions versus CIN3-like (HPV E4 negative but with extensive diffuse p16 expression) transforming CIN2 lesions.<sup>16–18</sup>

### Laboratory processing

FFPE cervical tissue blocks will be sectioned for the main analyses as follows: one section for H&E (2.5–3 μm), one for p16<sup>INK4a</sup> (3.5 μm), one for HPV E4 (3.5 μm), three sections (each of 8 μm) for HPV testing in a separate study, four unstained sections (each of 3.5 μm) for future analyses and one for H&E (2.5–3 μm) (figure 2). To avoid cross-contamination, redundant tissue material will be removed by extensive vacuum cleaning and cleaning with 1% SDS solution and ethanol between each block. New pencils and microtome knives will be applied between each section of block. One blank section will be cut after every 10th block to check for any cross-contamination of HPV. The sandwich technique will be applied to enable histopathological review of H&E slides flanking the sections subject for analysis. Immunohistochemical staining for intraepithelial p16<sup>INK4a</sup> reactions will be performed using CINtec Histology (Roche Diagnostics, Denmark), whereas staining for HPV E4 will be carried out using a pan HPV E4 biomarker; that is, the E4 SILgrade-E4 8XR-E4-1 (2 μg/mL) (DDL Diagnostic Laboratory, The Netherlands). This can detect E4 expression from a wide range of HPV genotypes. All immunohistochemical analyses will be performed on the Ventana BenchMark Ultra automated immune-stainer (Roche Diagnostics) following the manufacturer’s instructions. Negative controls (no primary antibody) and positive controls (multi-tissue-array blocks positive for p16<sup>INK4a</sup>) and (cervical samples (CIN1) positive for HPV E4) will be



**Figure 2** Microtome sectioning flow of tissue slides. H&E: tissue slides for hematoxylin and eosin (H&E) staining (start/end) (2×2.5–3 μm). p16<sup>INK4a</sup>: tissue slides for p16<sup>INK4a</sup> immunohistochemical staining analysis (1×3.5 μm). HPV E4: tissue slides for human papillomavirus (HPV) E4 immunohistochemical staining analysis (1×3.5 μm). Unstained slides for future analysis (4×3.5 μm). HPV-genotyping: tissue sections for HPV-genotyping (3×8 μm). \*Blank slides are provided between every 10th tissue block to check for any cross-contamination.

included in each run of the immunohistochemical analyses to validate the results.

### Grading of cervical lesions

The intraepithelial expression pattern of HPV E4 will be specified as the primary exposure variable of interest in addition to H&E morphology analysis and intraepithelial expression of p16<sup>INK4a</sup>. All CIN grading analyses will be performed by an international expert panel of four consultants with more than 20 years of experience in cervical pathology; one from the USA, one from the UK and two from Denmark.

Prior to the evaluation of slides, a structured scoring manual for evaluation of p16<sup>INK4a</sup> and HPV E4 staining will be prepared and tested by all pathologists to ensure an aligned base for all evaluators in the expert panel prior to the study start. Evaluation of slides will be based on a study-specific protocol describing the extent of biomarker epithelial staining. p16<sup>INK4a</sup> intraepithelial expression will be based on a two-tiered score system defined by the extent of reaction in the epithelial layer; negative or positive reaction with reference to the LAST criteria.<sup>4</sup> The intraepithelial reaction pattern of HPV E4 will be evaluated in the worst part of the cervical lesion and will be described by a two-tiered score system as positive or negative. Positive reactions will be defined as a strong reaction for HPV E4 with reference to the extent of dysplastic cells occupied in the epithelial layer. Negative HPV E4 reactions will be identified as none or sparse HPV E4 reaction in the epithelial layer. Evaluation of slides will be performed in the following order: (1) H&E; (2) H&E+p16<sup>INK4a</sup>; (3) H&E+p16<sup>INK4a</sup>+HPV E4. Initially, H&E morphology analysis will be performed by each pathologist independently and blinded from each other. Subsequently, p16<sup>INK4a</sup> and HPV E4 reaction patterns will be coevaluated as positive or negative by each pathologist supported by H&E morphology analysis, respectively. If inconclusive results are found, all assessors in the expert panel will meet and discuss the findings on a consensus meeting. One pathologist in the expert panel will be appointed as a major assessor with the authority to make the conclusion if inconsistent results are evident.

In the statistical analyses, a positive result (exposed) will be defined as an expression of an HPV E4-positive intraepithelial reaction. A negative result (unexposed)

will be defined as a negative HPV E4 intraepithelial reaction (table 1).

### Data management and statistical analysis

REDCap (Vanderbilt University 2021, hosted by the Department of Clinical Medicine, Aarhus University) will be used as electronic data capture platform for all data entry and data management.

For primary analysis, we will use the CIN2 community diagnosis, while the secondary analysis will be restricted to women in whom the CIN2 diagnosis is verified by the

**Table 1** Overview and main characteristics of the study

Study registration number	ClinicalTrials.gov NCT05049252
Location	Aarhus University Hospital, Central Denmark Region, Denmark
Study affiliation	Department of Gynecology and Obstetrics, NIDO - Center for Research and Education, Gødstrup Hospital, DK Department of Clinical Medicine, Aarhus University, DK Department of Pathology, Aarhus University Hospital, DK
Design	Historical cohort
Study period	2020–2023
Time of CIN2 diagnosis	2000–2010
Study population	N=500, women 23–40 years of age with an incidental CIN2 diagnosis, managed by active surveillance (2 years)
Database	The Danish Pathology Databank
Primary SNOMED codes	M74B09, T83110
Biomarkers	SILgrade-E4 8XR-E4-1 (2 ug/mL); p16 <sup>INK4a</sup> (CINTec, antibody clone E6H4)
Laboratory methods	Immunohistochemical staining (Ventana, BenchMark ULTRA, Roche Diagnostics)
Exposure	Positive HPV E4 intraepithelial expression
Outcome	Regression defined as CIN1 or less
Main variables considered in the statistical analysis	<ol style="list-style-type: none"> <li>1. Number of cervical punch biopsies collected at the time of index CIN2 community diagnosis.</li> <li>2. Number of follow-up visits during active surveillance.</li> <li>3. Age will be considered in an ancillary analysis</li> <li>4. Cervix cytology status at index CIN2 diagnosis in an ancillary analysis</li> </ol>
CIN, cervical intraepithelial neoplasia; HPV E4, Human papillomavirus E4 immunohistochemical biomarker; SNOMED, Systematized Nomenclature of Medicine.	

expert panel. In a separate study, we will describe the interobserver variation of CIN2 diagnosis, p16<sup>INK4a</sup> and HPV E4.

Women will contribute time at risk for the outcome from the time of incidental CIN2 diagnosis until the time of cone biopsy (T83701), hysterectomy (P306×0 or P30Y1), or end of follow-up whichever occurs first (online supplemental file 1). End of follow-up will be specified as 2 years and 4 months after incidental CIN2 diagnosis to take into account time from the last result of cervical punch biopsies to the time of a potential cone biopsy for those who had persistent CIN2 or CIN3 at the 2-year follow-up visit.

Results will be presented descriptively by tabulation of numbers (N) and proportions (%). For main statistical analyses, we will estimate the probability of regression among exposed (HPV E4 positive) versus non-exposed (HPV E4 negative), formally calculated as relative risks (RR) with corresponding 95% CIs. We will use a modified Poisson regression model, using robust variances to take into account potential confounding variables or modifying factors in relation to the association studied: age, result of the cervical cytology sample at the time of index CIN2 diagnosis, number of cervical biopsies collected at index CIN2 diagnosis and number of follow-up visits during the surveillance period. Results will be presented overall and stratified by age ( $\leq 30$  years vs  $> 30$  years). We will also report the median time from CIN2 diagnosis to time of the subsequent biopsies at last follow-up visit during active surveillance.

Based on the review of previous studies and clinical experience we expect an HPV E4 prevalence of 60% among women who subsequently regressed. To detect an effect size of RR=1.3 among HPV E4 exposure groups and regression, we estimate a statistical power of 86% at the sample size N=500 and significance level ( $\alpha$ )=0.05 (two-sided).

STATA V.15 (Stata Corp) will be used for all statistical analyses.

### Patient and public involvement statement

Patient and public involvement are not relevant.

### ETHICS AND DISSEMINATION

The study has been approved by the Danish Scientific Ethical Committee in Central Denmark Region<sup>1-10</sup> on 17 June 2020 and is registered at the Faculty of Health, Aarhus University as required by Danish legislation. Since biological material and data have already been collected from women prior to the study, the Danish Scientific Ethical Committee deemed it unnecessary to obtain written informed consent from participants in the study. All women registered in the Danish Tissue Availability Register (ie, women who oppose providing their tissue material for research purposes) will be excluded from the study, as required by Danish legislation.

The study results will be shared with the public and peers through publications in relevant international peer-reviewed scientific journals. Additionally, results will be presented at academic meetings, conferences, and to the public through press releases.

### PROJECT STATUS

Retrieval of data from the Danish Pathology Databank and collection of archived tissue samples started in December 2020 until January 2022. Subsequently, all tissue blocks will be sectioned and evaluated. Tissue sections from included women will subsequently be evaluated by the expert panel as previously described. Review process of tissue slides will take place from January 2022 through December 2022. Finally, data management and main data analyses are expected to be performed from December 2022 to July 2023.

### DISCUSSION

#### Present study and prior research

To our knowledge, this is the first cohort study to explore the use of the molecular biomarker HPV E4 as a risk marker for CIN2 evolution. Using archived tissue samples and high-quality registers we will examine the intraepithelial expression pattern of HPV E4 in cervical punch biopsies in young women diagnosed with CIN2 undergoing active surveillance. We will estimate the risk of regression by HPV E4 expression pattern (positive vs negative). Exploring whether HPV E4 may be useful for risk stratification of women diagnosed with CIN2 is of great clinical relevance as this information might allow for targeted treatment of women with low likelihood of regression and active surveillance of those with a high likelihood of regression. This information may be useful in clinical counselling when women are diagnosed with CIN2.

Few studies have previously examined the performance of HPV E4 for classification of precursor lesions of the cervix, either as a single biomarker or in combination with other relevant biomarkers.<sup>15-17</sup> These studies have demonstrated an inverse association between HPV E4 expression and grade of CIN lesion ( $p=0.001$ ).<sup>15-18</sup> Importantly, Griffin *et al* and van Baars *et al* showed that the HPV E4 biomarker could discriminate CIN2/CIN3 from CIN1/CIN2 lesions and that HPV E4 improved the interobserver agreement rate of CIN diagnoses compared with the p16<sup>INK4a</sup> biomarker alone.<sup>18</sup> Vink *et al* showed that HPV E4 expression in CIN3 was significantly higher in women  $< 29$  years versus those above 29 years of age indicating the relevance of age in the interpretation.<sup>16</sup> However, the primary focus in previous studies has been to examine the performance of the HPV E4 biomarker in relation to correct classification of CIN grade rather than estimating the likelihood of regression versus non-regression over time. This information may potentially allow a targeted treatment of women at the highest risk



of persistent disease or progression, thereby reducing the risk of undertreatment or overtreatment of women who would most likely regress. Furthermore, this information may reduce the psychological burden associated with active surveillance of CIN2, as reported in previous studies.<sup>37</sup>

### Strengths and limitations

Key strengths of this study are the unique possibility to explore CIN2 evolution using archived histological samples from a large cohort of young women in whom follow-up has been reported in high-quality nationwide registries. In contrast to previous studies on this subject,<sup>15–17</sup> risk of selection bias is considered minimal in the current study as active surveillance has been recommended in Central Denmark Region to women of reproductive age with a future childbearing desire since 1995. There are no restrictions (ie, lesion size, preceding cervical cytology result or risk factors). Thus, the randomly selected study population will be expected to reflect an unbiased sample from the source population of women with a record of CIN2. Since the purpose of the current study is to explore the use of biomarkers for risk stratification of CIN2 on a biological level, the results may be generalisable to other populations of women with CIN2 at similar age, screening history and follow-up strategy.

The use of an international expert group of four pathologists as independent evaluators of slides may result in increased generalisability of our findings. Another strength is that all women included in the cohort have initially been diagnosed in the same pathology department and not at multiple sites, which likely reduces variability in the assessment of samples.

We used regression models to estimate the risk of CIN2 regression over time, which may be useful in clinical counselling of women. In the study, we will only be able to include women with a record of subsequent cervical punch biopsies meaning that non-participating women will not be eligible. However, since primary screening, including subsequent clinical follow-up and treatment, has been well-organised for decades in Central Denmark Region, we expect to have a representative sample of women, and we do not expect the biomarker expression to be different from non-participating women with an undetected CIN2 diagnosis.

A limitation is the well-known low reproducibility associated with a CIN2 diagnosis. It is possible that some samples could have been misclassified based on the community diagnosis, however, in an ancillary analysis, we will restrict to cases in which the CIN2 diagnosis has been verified by the expert panel. Yet, misclassification may remain. In that case, this would be expected to go in both directions for all women in the study population and not cause differential misclassification of the results. Another limitation is the risk of detection bias, however, as Danish guidelines recommend collection of multiple biopsies, we expect the risk to be low. As we cannot guarantee that clinicians have adhered to guidelines, we will

take into account the number of biopsies in our statistical model. Unfortunately, we will not be able to assess whether knowledge of active surveillance might have affected the pathologist's community-based diagnosis, potentially resulting in downgrading or upgrading of lesions.

In this study, cervical punch biopsies will be used as the marker for the presence and grade of CIN or cancer. Unfortunately, we will not be able to determine whether cervical biopsies were collected from the worst part of the cervical lesion at baseline or during follow-up, as we have no information on colposcopic findings. Nor are we able to assess if biopsies were collected from the same location on the cervix as the original lesion. Nevertheless, this simply reflects clinical reality and current practice in colposcopy clinics. Clinical management of women depend on the result of biopsies collected at each follow-up visit during the active surveillance period. Thus, the median follow-up time and number of cervical punch biopsies taken will be expected to vary between women in the population studied. However, women in the study were assigned to exposure groups at baseline, and we do not expect to introduce any differential misclassification of the study results.

### Perspectives

A reliable diagnostic tool for risk stratification of CIN2 is of profound clinical importance. Strengthening risk assessment of CIN2 may reduce risk of overtreatment and undertreatment and allow for targeted treatment of women at increased risk of persistent disease and progression; and follow-up of those who would most likely regress. Thus, risk assessment performed at the time of incidental CIN2 diagnosis may be useful in clinical counselling and may strengthen the shared decision-making. As other countries are adopting active surveillance of younger women with CIN2, our study results may be useful for other countries as well.

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## Supplementary file 1

**Table S1. Displays relevant primary and secondary M, T and P SNOMED-codes used in the study.**

Primary SNOMED codes			
Moderate grade of dysplasia/CIN2	M74B09	-	-
Cervical punch biopsy	-	T83110	-
Secondary SNOMED codes			
	M-code	T-code	P-code
<b>Normal epithelial cells:</b>			
Normal tissue of cells	M0100	-	-
No tumor cells	M09460	-	-
Inflammatory changes	M4****(a)	-	-
Cellular changes due to inflammation	M69780	-	-
Unspecific reactiv cells	M01111	-	-
<b>Low grade of epithelial changes:</b>			
Low grade of dysplasia/CIN1	M74A09	-	-
CIN1 (non-keratinized type)	M74AB9	-	-
CIN1 (keratinized type)	M74AA9	-	-
Atypical squamous epithelial cells	M73005	-	-
Atypical cells	M69700	-	-
Atypical metaplasia	M73005	-	-
Koilocytosis	M69790	-	-
Condyloma	M76****(b)	-	-
Atypical glandular epithelial	M69762	-	-
<b>High grade of epithelial changes:</b>			
CIN2 (non-keratinized type)	M74BB9	-	-

CIN2 (keratinized type)	M74BA9	-	-
CIN2 (metaplastic origin)	M74BC9	-	-
High grade of dysplasia/CIN3	M74C09	-	-
CIN3 (non-keratinized type)	M74CB9	-	-
CIN3 (keratinized type)	M74CA9	-	-
CIN3 (metaplastic origin)	M74CC9	-	-
Planocellular carcinoma in situ (CIS)	M80*** <sup>(c)</sup>	-	-
Adeno carcinoma in situ (AIS)	M80702	-	-
Planocellular carcinoma cancer	M80703	-	-
Microinvasive planocellular carcinoma	M80763	-	-
Cone excisional biopsy	-	T83701	-
Hysterectomy	-	-	P306X0 and/or P30Y1
Cervix cytology	-	T8X310 or T8X312	-

a) \*\*\*\* defines all M-code subtypes for inflammation. b) \*\*\* describes M-code subtypes for condyloma.

c) \*\*\* defines M-code subtypes for CIS