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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039636
Article Type:	Protocol
Date Submitted by the Author:	21-Apr-2020
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Keywords:	EPIDEMIOLOGY, Cytopathology < PATHOLOGY, PREVENTIVE MEDICINE, PUBLIC HEALTH, GYNAECOLOGY

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EXPANDING THE UPPER AGE LIMIT FOR CERVICAL CANCER SCREENING: A PROTOCOL FOR A NATIONWIDE NON-RANDOMIZED INTERVENTION STUDY

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Number of words in text: 3,891

Number of words in abstract: 295

Number of figures: 3

Number of tables: 1

Number of references: 51

Trial registration: NCT04114968.
s:

Keywords: Mass screening, HPV DNA testing, older women, cervical cancer screening, cervical cancer

ABSTRACT

Introduction

Cervical cancer screening ceases between the ages of 60 and 65 in most countries. Yet, a relatively high proportion of cervical cancers are diagnosed in women above the screening age. This study will evaluate if expanding the upper screening age to include women aged 65-69 years results in increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to women not offered screening, and to determine if cervico-vaginal self-sampling for human papillomavirus (HPV self-sampling) is superior to general practitioner (GP)-based screening in reaching long-term unscreened women.

Methods

This population-based non-randomized intervention study will include 10,000 women aged 65-69 years, with no record of a cervical cytology sample or screening invitation in the 5 years before inclusion. Women who have opted-out of the screening program or have a record of hysterectomy or cervical amputation are excluded. Women residing in the Central Denmark Region are allocated to the intervention group, while women residing in the remaining four Danish regions are allocated to the reference group.

The intervention group is invited for HPV-based screening by attending routine screening at the GP or by requesting a self-sampling kit. The reference group receives standard care which is the opportunity to have a cervical cytology sample obtained at the GP or by a gynecologist. The study started in April 2019 and will run over the next 2.5 years.

Analyses

The primary outcome will be the proportion of CIN2+ detected in the intervention and reference groups.

Ethics and dissemination

The study has been submitted to the Ethical Committee which deemed that the study was not notifiable to the Committee and informed consent is therefore not required. The study is approved by the Danish Data

Protection Regulation and the Danish Patient Safety Authority. Results will be disseminated in peer-reviewed journals.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This population-based intervention study will provide new evidence on the effect of including women aged 65-69 years in organized cervical cancer screening
- This study is the first to evaluate if HPV self-sampling is superior to general practitioner-based screening in reaching long-term unscreened women aged 65-69 years
- The risk of information bias and selection problems are minimized by using high-quality Danish registries and a population-based design
- The study design entails a risk of confounding due to the lack of randomization

INTRODUCTION

Cervical cancer screening ceases between the ages of 60 and 65 in most Western countries (1-3). There is no solid evidence about which age and with which criteria to cease screening (1, 4-6), but the cessation of screening in older women is often justified by a low prevalence of high-risk human papillomavirus (HPV) in women ≥55 years (7, 8) and by a concern that the harms of continuing screening may outweigh potential benefits (2). Many countries with long-established screening programs, including Denmark, experience a second incidence peak of cervical cancer around the age of 75-80 years (9, 10). These older women are more often diagnosed with advanced-stage disease and mortality due to cervical cancer is high as compared to younger women (11, 12). It has been hypothesized that the incidence peak could be a result of a mid-life change of sexual partners or reactivation of a latent HPV infection as the immune system weakens with age (13-16). However, a recent Danish study of HPV DNA prevalence in women aged 69 and above showed no increase in prevalence that could explain the cervical cancer peak in this age group (17). It has also been hypothesized that the current peak in older ages could be attributed to an insufficient screening history in older birth cohorts (18). Whatever the reason, the increasing female life expectancy (at age 65 years it is about 20 additional years) has raised the question if the upper age limit for screening should be extended to 69 or 70 years (10, 19, 20). Case-control studies have reported benefits of cervical cytology screening at older ages with respect to reduced incidence and mortality (7, 21-24), even among previously screened women (4). However, a prospective evaluation of HPV-screening at ages 65-69 in a population-based intervention study including a reference group is missing.

The effectiveness of cervical cancer screening among older women will depend on the participation rate and, in particular, the ability to reach long-term unscreened women, as these women have a pronounced risk of cancer (6, 25). Currently, participation in routine screening decreases with increasing age leaving a relatively high proportion of older women under-screened (10). A potential solution to this challenge could be to offer older women a self-sampling kit for HPV testing (HPV self-sampling). HPV self-sampling is an accurate and well-accepted screening method, proven superior to physician-based screening in reaching

long-term unscreened women (26, 27). Yet, it remains unknown whether an older screening population will benefit from a self-sampling offer.

OBJECTIVES

This study will evaluate if expanding the upper screening age to include women aged 65-69-year results in increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to women not offered screening and will establish whether HPV self-sampling is better than general practitioner (GP)-based screening in reaching long-term unscreened women.

HYPOTHESES

We hypothesize that expanding the upper screening age will result in increased detection of CIN2+ cases and, long-term, potentially reduce the cervical cancer incidence compared to women not offered screening. Finally, we hypothesize that HPV self-sampling will be superior to GP-based screening in reaching long-term unscreened women.

METHODS AND ANALYSIS

Setting

Organized cervical cancer screening was introduced in parts of Denmark in the 1960s and became nationwide in the late 1990s (28, 29). Screening in Denmark is currently organized by the five regions (North, Central, South, Zealand, and Capital) following national guidelines (29, 30). Cervical cancer screening is centralized to one or a few pathology departments in each region (29). Danish women are invited to schedule an appointment with their GP for liquid-based cytology screening every third year when aged 23 to 49 years and every fifth year when aged 50 to 64 years(29). Since 2012, women aged 60-64 years have been screened with an HPV DNA-check-out test, after which HPV negative women can exit the program without consideration of their previous screening history (30). Outside the organized program, women can have a cervical cytology sample taken by a GP or a gynecologist opportunistically or due to clinical symptoms at any time. In Denmark, cervical cancer screening, including clinical follow-up and treatment, is free of charge (29).

The intervention in this study will be run by the Department of Public Health Programmes, Randers Regional Hospital in the Central Denmark Region (CDR). The CDR is the second largest region in Denmark covering approximately one-fourth of the Danish population (1.2 million inhabitants)(20). In the CDR, the Department of Public Health Programmes oversees sending screening invitations, reminders, and test results, while the Department of Pathology, Randers Regional Hospital handles and analyses all cervical cytology samples.

The call-recall invitation system

The Danish screening program is based on an integrated call-recall system using data from the invitation module in the nationwide Danish Pathology Databank (DPDB) (29, 31, 32). The Conseillers en Gestion et Informatique (CGI) Institute operates the call-recall system and it is designed so that each region only invites women residing in their catchment area. The call-recall system invites women for screening after the age-specific interval has passed since their latest invitation or cervical cytology sample (whichever came last). Samples obtained opportunistically, symptomatically or as part of surveillance are also recorded in the DPDB and postpone the next invitation. The system also keeps track of women who are ineligible for screening because they have actively opted out of the program or have had a hysterectomy. The latter registration is rather incomplete and varies between the regions. In detail, the invitation module links cervical cytology data (Systematized Nomenclature of Medicine, SNOMED codes: T8X3* and T8X210) from the DPDB's main pathology module with information about residency and vital status from the Danish Civil Registration System (33, 34). Linkage is performed using the unique Civil Personal Registration number (CPR), which is assigned to every Danish citizen upon birth and to residents upon immigration (34). The CPR number is used by all citizens for any contact to the Danish health care system.

Design and eligibility criteria

This study is a nationwide prospective population-based non-randomized intervention study (i.e. a quasi-experimental design) (35). Women will consecutively be deemed eligible if they meet the following criteria at the time of inclusion: aged 65 to 69 years; resident in Denmark for the past 5 years; no record of a cervical cytology sample or invitation in the past 5 years; not registered in the invitation module as having actively opted out of the screening program or having a record of total hysterectomy or cervical amputation in the Danish National Patient Register (36). Eligible women residing in the CDR will be allocated to the intervention group, while women residing in the other four Danish regions will be allocated to the reference group (Figure 1). In the intervention group, the invitation module will be set-up to identify

women fitting the inclusion criteria, and simultaneously a comparable list of eligible women in the reference group will be compiled by CGI at the Department of Public Health Programmes' request. Inclusion started in April 2019 and eligible women will be identified with six months intervals until the desired number of women have been included. The follow-up period for the included women will start on the date of the invitation and end at time of death, emigration, cervical amputation, total hysterectomy, or end of study. Women that move between the intervention region and reference regions in the follow-up period will subsequently be excluded from the analysis.

Intervention group

Women living in the CDR and therefore eligible for the intervention group will be invited to HPV-based cervical cancer screening by either scheduling an appointment for having a cervical cytology sample collected at their GP or collecting a cervico-vaginal sample themselves in their own home using a self-sampling kit. Women will receive an invitation and an information sheet by digital mail, while those exempted from digital mail as per routine will receive the information by postal mail (37). The invitation explains how to request the self-sampling kit and states that once the woman attends screening it will implicitly represent her consent to store her sample for future quality improvement of the screening program. A phone number for calling the study investigator to decline this option will be available. Test results, including follow-up recommendations, will be sent to the women by digital or postal mail and the woman's GP will receive an electronic copy of the test result. Around 98% of all residents in Denmark are listed with a GP (38). As per routine, non-participants will receive up to two reminders at 3 and 6 months post invitation (29). All information will be in Danish.

The self-sampling kit

The self-sampling kit can be requested by phone or through a study webpage. After receiving the orders in the department, the kit will be mailed to the women within four business days. The kit includes the dry Evalyn® brush self-sampling device (Rovers Medical Devices B.V, Oss, Netherlands) (39), written and

picture-based user instructions on how to collect and mail the self-sample, and a pre-stamped return envelope addressed to the Department of Pathology, Randers Regional Hospital (40). The acceptability of both the self-sampling device and user instructions has been carefully evaluated in previous studies, although among younger women (30-59 years) (41, 42).

Processing and analysis of samples

In the intervention group, all samples will be prepared, processed and analyzed at the Department of Pathology, Randers Regional Hospital according to the routine laboratory protocols. All HPV testing will be performed using the clinically validated and Federal Drug Agency (FDA)-approved Cobas® 4800 DNA test (Roche Diagnostics, Switzerland) (43), as this is the routine test platform used in the CDR. The test is an automated real-time PCR-based test designed to detect high-risk HPV types: 16,18,31,33,35,39,45,51,52, 56,58,59,66 and 68 (43, 44). Results will be reported as 1) HPV negative, 2) HPV positive (HPV16, HPV18 and/ or other HPV types) or 3) invalid (40). All samples with an invalid test result will be re-tested, and the second result will be considered definitive. The Cobas test measures beta-globin as an internal control for sample cellularity, valid sample extraction, and amplification (44).

As per routine, cervical cytology samples taken by the GPs will be collected using a cervical brush and rinsed in 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and mailed to the Department of Pathology, Randers Regional Hospital for processing and HPV testing. For HPV positive women, reflex cytology testing will be performed on the residual cellular Surepath material. Cytology will be interpreted by cytotechnologists using computer-assisted microscopy and categorized per the Bethesda 2015 grading system as normal; inadequate; Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H). High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC), and malignant tumor cells. At the laboratory, the Evalyn brush device will be rinsed into 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and processed as previously described (41).

From the cervical cytology samples and self-samples, 2 mL of the SurePath medium will be placed in test tubes for HPV testing. The residual eluate material from these samples will be stored at -80˚C for future extended genotyping and DNA methylation analysis. As part of the study, p16/Ki67 cytology dual staining (CINtec® PLUS cytology kit, Roche Diagnostics) will be performed consecutively on the residual SurePath cell-pellet obtained from women with an HPV positive cervical cytology sample and sufficient material for cytology testing. The dual staining result will not affect the clinical management of the woman. Except for the dual staining result, all test results will routinely be registered in the DPDB (32).

Clinical management

Figures 2 and 3 show the recommended, and therefore, expected clinical management for women in the intervention group, but management may deviate depending on the clinical presentation of the individual woman. The recommendations are in accordance with the routine screening guidelines for 60-64-year-old women and the new guidelines for clinical management of older women with dysplasia and HPV (30, 45). Women who are positive for HPV16 or 18 AND other types will be managed similar to HPV16/18 positive women.

For women attending GP-based screening, those who tested HPV negative will have no further follow-up (Figure 2). Women tested positive for HPV 16/18 will be referred directly to colposcopy (regardless of the cytology result). Women tested HPV positive for other types than HPV16/18 with ASC-US or more severe cytological abnormalities will be referred to colposcopy, while women with HPV types other than HPV16/18 and normal cytology will undergo repeated co-testing (HPV and cytology) after 12 months and will be referred for colposcopy if either test result is positive.

Figure 3 presents the follow-up recommendations for women attending HPV self-sampling. Women who tested HPV negative in their self-sample will have no further follow-up. Women with an HPV positive self-sample (any genotype) will be advised to have a cervical cytology triage sample taken by their GP within 30 days to evaluate the need for referral to colposcopy. This triage sample will be co-tested with HPV and cytology. Women tested HPV negative with normal cytology will have no further follow-up, while those

with ASC-US/LSIL cytology will undergo a repeat co-test (HPV and cytology) after 12 months and will be referred for colposcopy if either test result is positive. Those with ASC-H or more severe abnormalities will be referred for colposcopy. Women with an HPV positive triage cytology sample will follow the same recommendation as described for the GP-based screening (Figure 2).

For women referred for colposcopy, cervical punch biopsies will be taken from suspicious areas, supplemented with random biopsies according to Danish guidelines (46). Some women may also undergo a diagnostic conization as part of a clinical "See and Treat" study (47). Histological examination of the cervical biopsies will be carried out at different local Pathology Departments and graded using the Cervical Intraepithelial Neoplasia (CIN) classification as normal (including inflammation and non-specific reactive features), CIN (not specified), CIN grade 1, 2 or 3/AIS, or invasive cancer.

Reference group

Women in the reference group will receive usual care which, for 65-69-year-old women, is the opportunity to have a cervical cytology sample obtained at their GP or by a gynecologist for whatever reason. The women will not receive a screening invitation, but will be assigned individual pseudo screening invitation dates allowing comparison between the groups in our statistical analysis. In the following, the term "invitation date" will be used for both for the "true invitation dates" in the intervention group and "pseudo invitation dates" in the reference group. In all reference regions, samples from this age group are expected to be tested for HPV. Differences across the four regions are found in the HPV assay (17) and there may be minor differences in the triage-strategies, which may result in differences in indication for colposcopy referral. However, clinical management of women referred for colposcopy is expected to follow national guidelines as described above (45, 46).

Outcomes

In both groups, the primary outcome will be the proportion of CIN2+ (CIN2, CIN3/AIS, and cancer) detected within 6 months following registration of a cervical cytology sample or self-sample. The proportion of CIN3+ (CIN3/AIS, and cancer) will be a secondary outcome. The most severe histological test result will be used if more than one result is available in the follow-up period. Other outcomes in the intervention group will be screening participation measured 12 months after the invitation date, defined by returning a self-sample or having a cervical cytology taken; screening history of participants, stratified by sampling procedure; HPV positivity rate and HPV type distribution in self-samples versus GP-collected cervical cytology samples; and the percentage of HPV positive self-samplers undergoing appropriate follow-up. Follow-up after self-sampling will be defined as attending a GP for a cervical cytology-triage sample within 30, 60, 90 or 180 days after mailing of the test results. The proportion of cervical cytology samples obtained among women not invited for screening will be identified in the reference group and measured 12 months post invitation date. As in another study (2), the primary measure of harms will in both groups be the number of colposcopies/conizations performed, both overall and relatively to CIN2, CIN3/AIS, and cancers detected within a follow-up period of 6 months after registration of a cervical cytology sample or self-sample. Long-term outcomes will be cervical cancer incidence rates reported by groups at 5- and 10-years post invitation dates. A description of histological type and FIGO stage of the detected cervical cancers will be provided.

Data sources and statistical analysis

An overview of data sources and information is seen in Table 1.

Baseline characteristics in both groups will be presented using descriptive statistics (numbers and proportions) on screening history and sociodemographic factors (e.g. age, marital status, and education level). Screening history will be categorized based on the woman's screening history in a 15-year period before screening exit (i.e. age 50-64) according to the results of the cytology screening at age 50-59 and the HPV-exit test at age 60-64. The categorization of screening history is expected to be as follows (6):

1) "Sufficiently screened with normal results" if women had a) at least one normal cytology at age 50-54 and b) at least one normal cytology at age 55-59, and c) no abnormal cytology (ASC-US or worse) at age 50-59, and d) HPV negative at age 60-64; 2) "Insufficiently screened with normal results" if women had one or more cytology samples with only normal results, but only in one or two age categories (50-54, 55-59 or 60-64); 3) "Long-term unscreened" if no cervical cytology sample at age 50-64; and 4) "Abnormal screening" if women a) had ASC-US or worse at least once at age 50-59 or b) HPV positive at age 60-64.

Screening participation, cervical cytology samples, numbers of colposcopies/conizations performed, compliance to follow-up among positive self-samplers, and disease outcomes (HPV positivity rate and histological outcomes) will be estimated as proportions. Participation in the intervention group will be reported by age groups, screening history, and sampling method (GP versus self-sampling). Regression analyses will be used to estimate the association between CIN2+ detection in women offered cervical cancer screening compared to those not offered screening. Both crude and adjusted estimates will be presented with 95% confidence intervals (CIs). The cumulative incidence rates of cervical cancer among women in the intervention and reference groups will be reported, including the distribution of the histological types and FIGO stage of the detected cancers. All statistical analyses will be performed using STATA version 16 (College Station, TX: StataCorp LP).

Sample size

The sample size was determined by the primary outcome (CIN2+). In Denmark, approximately 167,000 Danish women are currently aged 65-69 years, 37,000 of whom (22,2%) reside in the CDR (intervention group) (20). We anticipate that an estimated 55% of these will not have a record of a cervical cytology sample in the DPDB within the past five years. As follows, the study cohort will consist of a total of 91,850 women, including approximately 20,000 women in the intervention group. We assume that 50% of the eligible women in the intervention group will accept the screening offer and that the proportion of CIN2+ is 0.3% among participants. Thus, by including 10,000 women in the intervention group, the study will have a power of 80% to detect a difference in the CIN2+ proportion of 0.1 percentage points between the

intervention and reference group. The proportion of CIN2+ that was chosen (0.3%) is a conservative estimate inspired by Swedish data reporting a CIN2+ proportion of 0.38% among 56-60-year-old women attending HPV-based screening using the Cobas 4800 test (48).

Timeline

The study enrollment is expected to continue until 10,000 participants have been included in the intervention group. Invitations will be sent out prospectively over an expected 2.5 year-period starting April 2019.

PATIENT AND PUBLIC INVOLVEMENT

The research questions were developed in response to the on-going public and scientific discussion in Denmark regarding expanding the upper screening age in the organized cervical cancer screening program. No patients or patient organizations were involved in the development, design, or implementation of this study.

ETHICS AND DISSEMINATION

Ethics

According to the EU's General Data Protection Regulation, the project was listed at the record of processing activities for research projects in the CDR (j. no: 1-16-02-158-18). The study was approved by the Danish Patient Safety Authority (j.no: 3-3013-2634/1). The study protocol has been submitted to the Ethical Committee in the CDR. The Committee decided that according to the Consolidation Act on Research Ethics Review of Health Research Projects, Consolidation Act number 1083 of 15 September 2017 section 2 (1), this study is not notifiable to the Committee (j.no.: 73/2018) and informed consent is therefore not required.

Dissemination

The study protocol is registered at ClinicalTrials.gov (NCT04114968) and is made public in this protocol article. The results will be reported through publication of peer-reviewed articles in international scientific journals and presented at national and international scientific meetings. Moreover, the study results will be disseminated to healthcare stakeholders, and patient organizations at scientific meetings, and to the general public through press releases.

STRENGTHS AND LIMITATIONS OF THIS STUDY

To our knowledge, this prospective population-based intervention study will be the first to evaluate if HPV-based cervical cancer screening among older women aged 65-69 years results in an increased detection of CIN2+ cases as compared to women not offered screening. Importantly, this study will evaluate whether the potential harms of overtreatment in older women are outweighed by the potential benefits of decreasing the incidence of (pre)-cancer (2, 49). Overall, this knowledge will address important research gaps and guide future screening recommendations. Compared with previous studies which report, by necessity, only the effect of cytology screening at older ages (7, 21-24) it is of great value for future decision making that this study will be able to determine the effect of screening at older ages in women who have had an exit HPV-test (49). A key strength is that the effect of the screening intervention will be measured prospectively within an organized program. From an implementation point of view, this will provide reliable estimates of the expected participation rates if extending the upper screening age together with the possibility of self-sampling would become routine. We will identify outcomes from the nationwide DPDB which has highly valid records on all pathology specimens in Denmark (32), and the selection of study participants is population-based and determined by data from the invitation module; thus eliminating both information bias and selection problems. Important limitations should be mentioned. The lack of randomization gives rise to confounding of both known and unknown risk factors. Ideally, eligible women in all Danish regions should have been individually randomized to the intervention and reference group instead of being allocated to the groups based on their geographical location. Unfortunately, this was not feasible from an organizational point of view. Detection of invasive and advanced cervical cancer is the optimal outcome measure to evaluate the effect of screening at older ages (49), but given the relative rarity of cervical cancer in older women, the length of follow-up needed and the large sample size required, we chose CIN2+ and CIN3+ as the primary and secondary outcome, respectively.

Yet, it should be noted that the majority of CIN2 and CIN3 lesions detected after age 65 might not have

sufficient time to progress to invasive cancer in the remaining lifespan (2). However, the inclusion of CIN2/3 cases is justified by them being treatable endpoints in older non-reproductive women (45).

Trial status

Ongoing.

Acknowledgements:

Not applicable.

Contributors:

MT is the principal investigator of the study and is responsible for conducting the study overall. BA and MT conceived the original idea. Subsequently, LKP, ME, AH, MBH and JB also contributed to the design of the study. JSJ especially contributed with comments on the laboratory part of the protocol, while LKP, JB, and AH have provided clinical advice on follow-up of women with abnormal results. MT is the first author and drafted the first version of this protocol article, which was subsequently further developed by all authors, who also reviewed and approved the final version.

Funding:

The study was funded by the Health Foundation. The funding body had no role in the design of the study and collection, analysis, and interpretation of the data and in writing the manuscript.

Competing interests:

Roche sponsors the Cobas HPV-DNA test kits and CINtec Plus test kits for the study. According to the contract between Roche and the Department of Public Health Programmes, Randers Regional Hospital, Roche has commented on the protocol article, but had no influence on the scientific process and no editorial rights pertaining to this manuscript. The authors retained the right to submit the manuscript. MT, JB, JSJ and BA have participated in other studies with HPV test kits sponsored by Roche and self-sampling devices sponsored by Axlab. MT has received honoraria from Roche Diagnostics and AstraZeneca for lectures on HPV self-sampling and HPV triage-methods, respectively. AH has received lecture fees from AstraZeneca. All authors declare no conflicts of interest.

Patient consent:

Not required

Ethics approval:

The study protocol has been submitted to the Ethical Committee which deemed that the study was not notifiable to the Committee and informed consent is therefore not required.

Provenance and peer review:

Not commissioned; externally peer-reviewed.

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Table 1: Overview over data sources and information

Data sources	Information
Danish Pathology Data Bank (32)	Participation (yes/no) Participation by self-sampling or GP-based screening Cervical cytology samples in references regions Results of self-samples, cervical cytology samples and cervical biopsies Cervical biopsy performed (yes/no) Conization performed (yes/no) Screening history
Danish Civil Registration System (34)	Residence Date of death, emigration and immigration
Danish National Patient Register (36)	Total hysterectomy and cervical amputation procedures
Danish Cancer Registry (50)	Cervical cancer incidence
Statistics Denmark (51)	Sociodemographic factors (e.g. age, marital status and education level)

Table notes: GP: General Practitioner.

Figure 1: Map of the intervention and reference regions

Figure 2: Clinical management of women attending screening at a GP

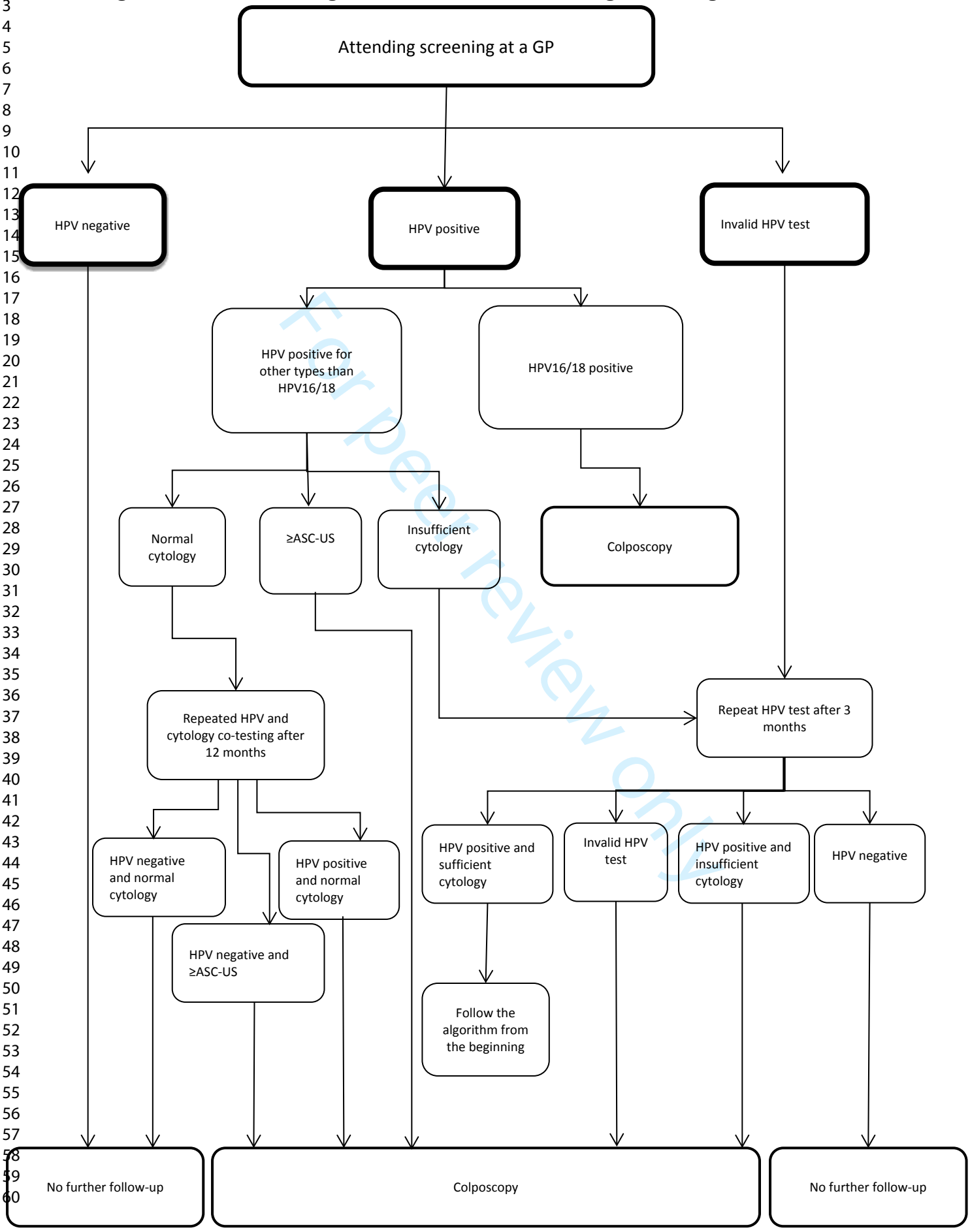

Figure notes: GP: General Practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US includes: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H); High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC) and malignant tumor cells.

Figure :3 Clinical management of women attending HPV self-sampling

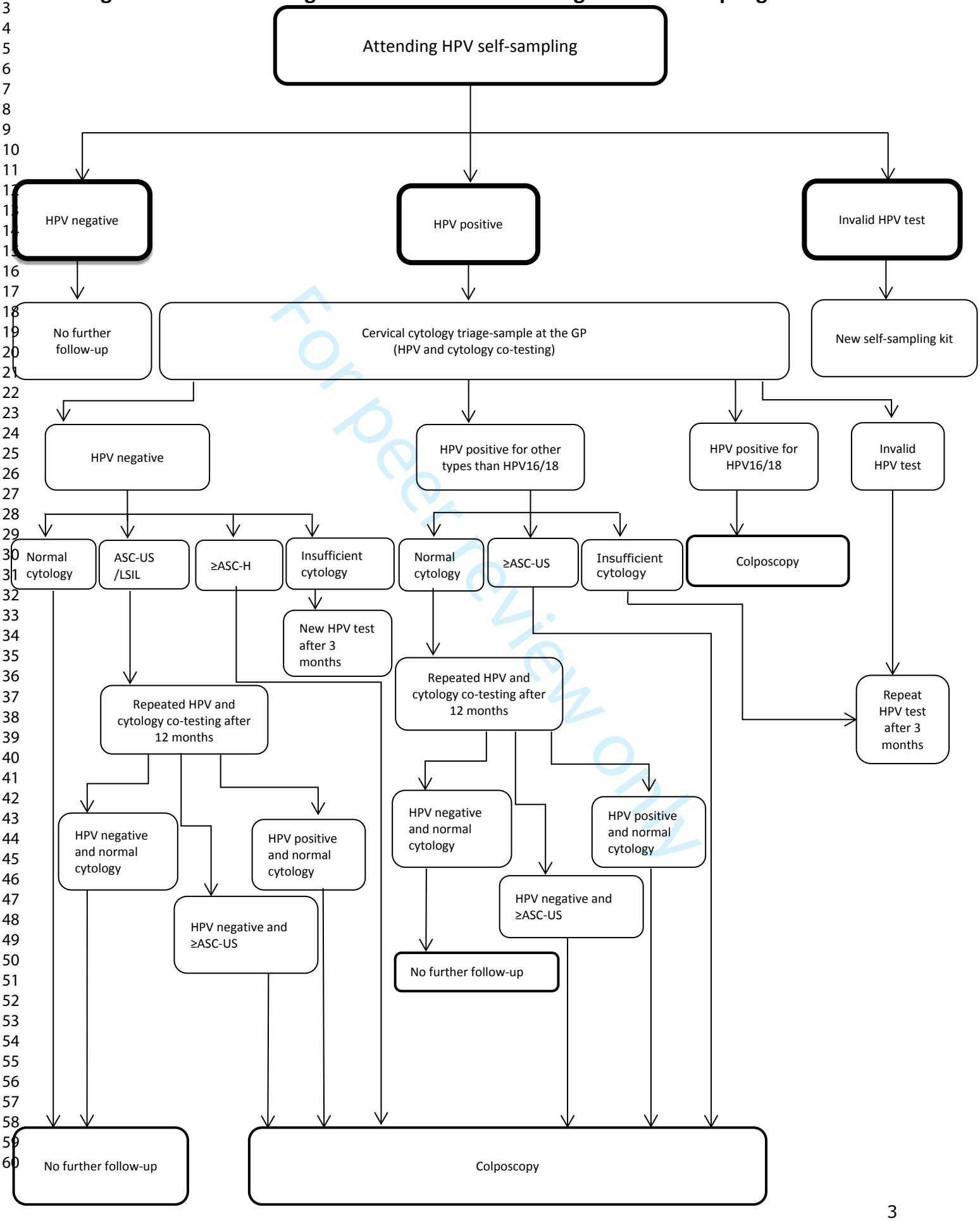

Figure notes: GP: General practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US include: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H); High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC) and malignant tumor cells. ≥ASC-H include: ASC-H, HSIL, SCC, AGC, AIS, ACC, and malignant tumor cells.

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EXPANDING THE UPPER AGE LIMIT FOR CERVICAL CANCER SCREENING: A PROTOCOL FOR A NATIONWIDE NON-RANDOMIZED INTERVENTION STUDY

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039636.R1
Article Type:	Protocol
Date Submitted by the Author:	14-Jul-2020
Complete List of Authors:	tranberg, mette; Regional Hospital Randers, Department of Public Health Programmes Petersen, Lone; Odense University Hospital, Department of Obstetrics and Gynecology; University of Southern Denmark, OPEN, Department of Clinical Medicine Elfström, Klara; Karolinska Institutet, Dept of Laboratory Medicine; Regional Cancer Center, Cancer Screening Unit Hammer, Anne ; Aarhus University, Department of Clinical Medicine; Regionshospitalet Herning, Department of Obstetrics and Gynecology Blaakær, Jan; University of Southern Denmark, OPEN, Department of Clinical Medicine; Odense University Hospital, Department of Obstetrics and Gynecology Bennetsen, Mary; Regional Hospital Randers, Department of Pathology Jensen, Jørgen; Statens Serum Institut, Mycoplasma Laboratory Andersen, Berit; Randers Regional Hospital, Public Helath Programs; Aarhus University, Department of Clinical Medicine
Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology, Obstetrics and gynaecology
Keywords:	EPIDEMIOLOGY, Cytopathology < PATHOLOGY, PREVENTIVE MEDICINE, PUBLIC HEALTH, GYNAECOLOGY

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EXPANDING THE UPPER AGE LIMIT FOR CERVICAL CANCER SCREENING: A PROTOCOL FOR A NATIONWIDE NON-RANDOMIZED INTERVENTION STUDY

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Number of words in text: 4,121

Number of words in abstract: 324

Number of figures: 3

Number of tables: 2

Number of references: 54

Trial registration: NCT04114968.

Keywords: Mass screening, HPV DNA testing, older women, cervical cancer screening, cervical cancer

ABSTRACT

Introduction

Cervical cancer screening ceases between the ages of 60 and 65 in most countries. Yet, a relatively high proportion of cervical cancers are diagnosed in women above the screening age. This study will evaluate if screening of women aged 65-69 years may result in increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to women not invited to screening. Invited women may choose between general practitioner (GP)-based screening or cervico-vaginal self-sampling. Furthermore, the study will assess if self-sampling is superior to GP-based screening in reaching long-term unscreened women.

Methods and Analysis

This population-based non-randomized intervention study will include 10,000 women aged 65-69 years, with no record of a cervical cytology sample or screening invitation in the 5 years before inclusion. Women who have opted-out of the screening program or have a record of hysterectomy or cervical amputation are excluded. Women residing in the Central Denmark Region are allocated to the intervention group, while women residing in the remaining four Danish regions are allocated to the reference group.

The intervention group is invited for human papillomavirus (HPV)-based screening by attending routine screening at the GP or by requesting a self-sampling kit. The reference group receives standard care which is the opportunity to have a cervical cytology sample obtained at the GP or by a gynecologist. The study started in April 2019 and will run over the next 4.5 years.

The primary outcome will be the proportion of CIN2+ detected in the intervention and reference groups. In the intervention group, the proportion of long-term unscreened women attending GP-based screening or self-sampling will be compared.

Ethics and dissemination

The study has been submitted to the Ethical Committee in the Central Denmark Region which deemed that the study was not notifiable to the Committee and informed consent is therefore not required. The study is

approved by the Danish Data Protection Regulation and the Danish Patient Safety Authority. Results will be disseminated in peer-reviewed journals.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This population-based intervention study is the first to evaluate if expanding the upper screening age to include women aged 65-69-year and inviting them to choose between GP-based screening or self-sampling will result in increased detection of CIN2+ compared to existing practice (i.e. no screening).
- This study is the first to evaluate if self-sampling is superior to GP-based screening in reaching long-term unscreened women aged 65-69 years
- The risk of information bias and selection problems are minimized by using high-quality Danish registries and a population-based design
- The study design entails a risk of confounding due to the lack of randomization

INTRODUCTION

Cervical cancer screening ceases between the ages of 60 and 65 in most Western countries [1-3]. There is no solid evidence about which age and with which criteria to cease screening [1 4-6], but the cessation of screening in older women is often justified by a low prevalence of high-risk human papillomavirus (HPV) in women ≥55 years [7 8] and by a concern that the harms of continuing screening may outweigh potential benefits [2]. Many countries with long-established screening programs, including Denmark, experience a second incidence peak of cervical cancer around the age of 75-80 years [9 10], with a hysterectomy-corrected incidence rate of 29.4 per 100,000 person-years in women aged 75-79 [11]. These older women are more often diagnosed with advanced-stage disease and mortality due to cervical cancer is high as compared to younger women [12 13]. It has been hypothesized that the incidence peak could be a result of a mid-life change of sexual partners or reactivation of a latent HPV infection as the immune system weakens with age [14-17]. However, a recent Danish study of HPV DNA prevalence in women aged 69 and above showed no increase in prevalence that could explain the cervical cancer peak in this age group [18]. It has also been hypothesized that the current peak in older ages could be attributed to an insufficient screening history in older birth cohorts [19]. Whatever the reason, the increasing female life expectancy (at age 65 years it is about 20 additional years) has raised the question if the upper age limit for screening should be extended to 69 or 70 years [10 20 21]. Case-control studies have reported benefits of cervical cytology screening at older ages with respect to reduced incidence and mortality [7 22-25], even among previously screened women [4]. However, a prospective evaluation of HPV-screening at ages 65-69 in a population-based intervention study including a reference group is missing.

The effectiveness of cervical cancer screening among older women will depend on the participation rate and, in particular, the ability to reach long-term unscreened women, as these women have a pronounced risk of cancer [6 26]. Currently, participation in routine screening decreases with increasing age leaving a relatively high proportion of older women under-screened [10]. A potential solution to this challenge could be to offer older women a self-sampling kit for HPV testing (self-sampling). Self-sampling is an accurate and

well-accepted screening method, proven superior to physician-based screening in reaching long-term unscreened women [27 28]. Yet, it remains unknown whether an older screening population will benefit from a self-sampling offer.

OBJECTIVES

This study will evaluate if expanding the upper screening age to include women aged 65-69-year and inviting them to choose between general practitioner (GP)-based screening or self-sampling results in increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to existing practice where women in this age group of are not invited to routine screening . Furthermore, it will be assessed whether self-sampling is better than GP-based screening in reaching long-term unscreened women.

HYPOTHESES

We hypothesize that expanding the upper screening age will result in increased detection of CIN2+ cases and, long-term, potentially reduce the cervical cancer incidence compared to women not invited to screening. Finally, we hypothesize that self-sampling will be superior to GP-based screening in reaching long-term unscreened women.

METHODS AND ANALYSIS

Setting

Organized cervical cancer screening was introduced in parts of Denmark in the 1960s and became nationwide in the late 1990s [29 30]. Screening in Denmark is currently organized by the five regions (North, Central, South, Zealand, and Capital) following national guidelines [30 31]. Cervical cancer screening is centralized to one or a few pathology departments in each region [30]. Danish women are invited to schedule an appointment with their GP for liquid-based cytology screening every third year when aged 23 to 49 years and every fifth year when aged 50 to 64 years[30]. Since 2012, women aged 60-64 years have been screened with an HPV DNA-check-out test, after which HPV negative women can exit the program without consideration of their previous screening history [31]. Outside the organized program, women can have a cervical cytology sample taken by a GP or a gynecologist opportunistically or due to clinical symptoms at any time. In Denmark, cervical cancer screening, including clinical follow-up and treatment, is free of charge [30].

The intervention in this study will be run by the Department of Public Health Programmes, Randers Regional Hospital in the Central Denmark Region (CDR). The CDR is the second largest region in Denmark covering approximately one-fourth of the Danish population (1.2 million inhabitants)[21]. In the CDR, the Department of Public Health Programmes oversees sending screening invitations, reminders, and test results, while the Department of Pathology, Randers Regional Hospital handles and analyses all cervical cytology samples.

The call-recall invitation system

The Danish screening program is based on an integrated call-recall system using data from the invitation module in the nationwide Danish Pathology Databank (DPDB) [30 32 33]. The Conseillers en Gestion et Informatique (CGI) Institute operates the call-recall system and it is designed so that each region only invites women residing in their catchment area. The call-recall system invites women for screening after the age-specific interval has passed since their latest invitation or cervical cytology sample (whichever came last). Samples obtained opportunistically, symptomatically or as part of surveillance are also recorded in the DPDB and postpone the next invitation. The system also keeps track of women who are ineligible for screening because they have actively opted out of the program or have had a hysterectomy. The latter registration is rather incomplete and varies between the regions. In detail, the invitation module links cervical cytology data (Systematized Nomenclature of Medicine, SNOMED codes: T8X3* and T8X210) from the DPDB's main pathology module with information about residency and vital status from the Danish Civil Registration System [34 35]. Linkage is performed using the unique Civil Personal Registration number (CPR), which is assigned to every Danish citizen upon birth and to residents upon immigration [35]. The CPR number is used by all citizens for any contact to the Danish health care system.

Design and eligibility criteria

This study is a nationwide prospective population-based non-randomized intervention study (i.e. a quasi-experimental design) [36]. Women will consecutively be deemed eligible if they meet the following criteria at the time of inclusion: aged 65 to 69 years; resident in Denmark for the past 5 years; no record of a cervical cytology sample or invitation in the past 5 years; not registered in the invitation module as having actively opted out of the screening program or having a record of total hysterectomy or cervical amputation in the Danish National Patient Register [37]. Eligible women residing in the CDR will be allocated to the intervention group, while women residing in the other four Danish regions will be allocated to the reference group (Figure 1). In the intervention group, the invitation module will be set-up to identify women fitting the

inclusion criteria, and simultaneously a comparable list of eligible women in the reference group will be compiled by CGI at the Department of Public Health Programmes' request. Inclusion started in April 2019 and eligible women will be identified with six months intervals until the desired number of women have been included. The follow-up period for the included women will start on the date of the invitation and end at time of death, emigration, cervical amputation, total hysterectomy, or end of study. Women that move between the intervention region and reference regions in the follow-up period will subsequently be excluded from the analysis.

Intervention group

Women living in the CDR and therefore eligible for the intervention group will be invited to HPV-based cervical cancer screening by either scheduling an appointment for having a cervical cytology sample collected at their GP or collecting a cervico-vaginal sample themselves in their own home using a self-sampling kit. Women will receive an invitation and an information sheet by digital mail, while those exempted from digital mail as per routine will receive the information by postal mail [38]. The invitation explains how to request the self-sampling kit and states that once the woman attends screening it will implicitly represent her consent to store her sample for future quality improvement of the screening program. A phone number for calling the study investigator to decline this option will be available. Test results, including follow-up recommendations, will be sent to the women by digital or postal mail and the woman's GP will receive an electronic copy of the test result. Around 98% of all residents in Denmark are listed with a GP [39]. As per routine, non-participants will receive up to two reminders at 3 and 6 months post invitation [30]. All information will be in Danish.

The self-sampling kit

The self-sampling kit can be requested by phone or through a study webpage. After receiving the orders in the department, the kit will be mailed to the women within four business days. The kit includes the dry Evalyn® brush self-sampling device (Rovers Medical Devices B.V, Oss, Netherlands) [40], written and picture-

based user instructions on how to collect and mail the self-sample, and a pre-stamped return envelope addressed to the Department of Pathology, Randers Regional Hospital [41]. The acceptability of both the self-sampling device and user instructions has been carefully evaluated in previous studies, although among younger women (30-59 years) [42 43].

Processing and analysis of samples

In the intervention group, all samples will be prepared, processed and analyzed at the Department of Pathology, Randers Regional Hospital according to the routine laboratory protocols. All HPV testing will be performed using the clinically validated and Federal Drug Agency (FDA)-approved Cobas® 4800 DNA test (Roche Diagnostics, Switzerland) [44], as this is the routine test platform used in the CDR. The test is an automated real-time PCR-based test designed to detect high-risk HPV types: 16,18,31,33,35,39,45,51,52, 56,58,59,66 and 68 [44 45] and is validated for use on SurePath collected samples [46]. Results will be reported as 1) HPV negative, 2) HPV positive (HPV16, HPV18 and/ or other HPV types) or 3) invalid [41]. All samples with an invalid test result will be re-tested, and the second result will be considered definitive. The Cobas test measures beta-globin as an internal control for sample cellularity, valid sample extraction, and amplification [45].

As per routine, cervical cytology samples taken by the GPs will be collected using a cervical brush and rinsed in 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and mailed to the Department of Pathology, Randers Regional Hospital for processing and HPV testing. For HPV positive women, reflex cytology testing will be performed on the residual cellular Surepath material. Cytology will be interpreted by cytotechnologists using computer-assisted microscopy and categorized per the Bethesda 2014 grading system as normal; inadequate; Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H). High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC), and malignant tumor cells. At the laboratory, the

Evalyn brush device will be rinsed into 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and processed as previously described [42].

From the cervical cytology samples and self-samples, 2 mL of the SurePath medium will be placed in test tubes for HPV testing. The residual eluate material from these samples will be stored at -80˚C for future extended genotyping and DNA methylation analysis. As part of the study, and only in the intervention group, p16/Ki67 cytology dual staining (CINtec® PLUS cytology kit, Roche Diagnostics, Switzerland) will be performed consecutively on the residual SurePath cell-pellet obtained from women with an HPV positive cervical cytology sample and sufficient material for cytology testing. The dual staining result will not affect the clinical management of the woman. Except for the dual staining result, all test results will routinely be registered in the DPDB [33].

Clinical management

Figures 2 and 3 show the recommended, and therefore, expected clinical management for women in the intervention group, but management may deviate depending on the clinical presentation of the individual woman. The recommendations are in accordance with the routine screening guidelines for 60-64-year-old women and the new guidelines for clinical management of older women with dysplasia and HPV [31 47].

Women who are positive for HPV16 or 18 AND other types will be managed similar to HPV16/18 positive women.

For women attending GP-based screening, those who tested HPV negative will have no further follow-up (Figure 2). Women tested positive for HPV 16/18 will be referred directly to colposcopy (regardless of the cytology result). Women tested HPV positive for other types than HPV16/18 with ASC-US or more severe cytological abnormalities will be referred to colposcopy, while women with HPV types other than HPV16/18 and normal cytology will undergo repeated co-testing (HPV and cytology) after 12 months and will be referred for colposcopy if either test result is positive.

Figure 3 presents the follow-up recommendations for women attending self-sampling. Women who tested HPV negative in their self-sample will have no further follow-up. Women with an HPV positive self-sample

(any genotype) will be advised to have a cervical cytology triage sample taken by their GP within 30 days to evaluate the need for referral to colposcopy. This triage sample will be co-tested with HPV and cytology. Women tested HPV negative with normal cytology will have no further follow-up, while those with ASC-US/LSIL cytology will undergo a repeat co-test (HPV and cytology) after 12 months and will be referred for colposcopy if either test result is positive. Those with ASC-H or more severe abnormalities will be referred for colposcopy. Women with an HPV positive triage cytology sample will follow the same recommendation as described for the GP-based screening (Figure 2).

For women referred for colposcopy, cervical punch biopsies will be taken from suspicious areas, supplemented with random biopsies according to Danish guidelines [48]. Some women may also undergo a diagnostic conization as part of a clinical "See and Treat" study [49]. Histological examination of the cervical biopsies will be carried out at different local Pathology Departments and graded using the Cervical Intraepithelial Neoplasia (CIN) classification as normal (including inflammation and non-specific reactive features), CIN (not specified), CIN grade 1, 2 or 3/AIS, or invasive cancer.

Reference group

Women in the reference group will receive usual care which, for 65-69-year-old women, is the opportunity to have a cervical cytology sample obtained at their GP or by a gynecologist for whatever reason. The women will not receive a screening invitation, but will be assigned individual pseudo screening invitation dates allowing comparison between the groups in our statistical analysis. In the following, the term "invitation date" will be used for both for the "true invitation dates" in the intervention group and "pseudo invitation dates" in the reference group. In all reference regions, samples from this age group are expected to be tested for HPV. Differences across the four regions are found in the HPV assay [18] and there may be minor differences in the triage-strategies, which may result in differences in indication for colposcopy referral. However, clinical management of women referred for colposcopy is expected to follow national guidelines as described above [47 48].

Outcomes

Overview of outcomes and planned comparisons is seen in Table 1.

In both groups, the primary outcome will be the proportion of CIN2+ (CIN2, CIN3/AIS, and cancer) detected within 18 months following registration of a cervical cytology sample or self-sample. The proportion of CIN3+ (CIN3/AIS, and cancer) will be a secondary outcome. The most severe histological test result will be used if more than one result is available in the follow-up period. Other outcomes in the intervention group will be screening participation measured 12 months after the invitation date, defined by returning a self-sample or having a cervical cytology taken; screening history of participants, stratified by sampling procedure; HPV positivity rate and HPV type distribution in self-samples versus GP-collected cervical cytology samples; cytology results, and the percentage of HPV positive self-samplers undergoing appropriate follow-up. Compliance to follow-up after self-sampling will be defined as attending a GP for a cervical cytology-triage sample within 30, 60, 90 or 180 days after mailing of the test results. The proportion and results of cervical cytology samples obtained among women not invited for screening will be identified in the reference group and measured 12 months post invitation date. As in another study [2], the primary measure of harms will in both groups be the number of colposcopies/conizations performed, both overall and relatively to ≤CIN1, CIN2, CIN3/AIS, and cancers detected within a follow-up period of18 months after registration of a cervical cytology sample or self-sample. Long-term outcomes will be cervical cancer incidence rates reported by groups at 5- and 10-years post invitation dates. A description of histological type and FIGO stage of the detected cervical cancers will be provided.

Data sources and statistical analysis

An overview of data sources and information is seen in Table 2.

Baseline characteristics in both groups will be presented using descriptive statistics (numbers and proportions) on screening history, comorbidities, sociodemographic factors (e.g. age, marital status, and education level). Screening history will be categorized based on the woman's screening history in a 15-year period before screening exit (i.e. age 50-64) according to the results of the cytology screening at age 50-59

and the HPV-exit test at age 60-64. The categorization of screening history is expected to be as follows [6]: 1) "Sufficiently screened with normal results" if women had a) at least one normal cytology at age 50-54 and b) at least one normal cytology at age 55-59, and c) no abnormal cytology (ASC-US or worse) at age 50-59, and d) HPV negative at age 60-64; 2) "Insufficiently screened with normal results" if women had one or more cytology samples with only normal results, but only in one or two age categories (50-54, 55-59 or 60-64); 3) "Long-term unscreened" if no cervical cytology sample at age 50-64; and 4) "Abnormal screening" if women a) had ASC-US or worse at least once at age 50-59 or b) HPV positive at age 60-64.

Screening participation, cervical cytology samples, numbers of colposcopies/conizations performed, compliance to follow-up among positive self-samplers, and disease outcomes (HPV positivity rate and histological outcomes) will be estimated as proportions. Participation in the intervention group will be reported by age groups, screening history, and sampling method (GP versus self-sampling). Regression analyses will be used to estimate the association between CIN2+ detection in women offered cervical cancer screening compared to those not offered screening. Both crude and adjusted estimates will be presented with 95% confidence intervals (CIs).The cumulative incidence rates of cervical cancer among women in the intervention and reference groups will be reported, including the distribution of the histological types and FIGO stage of the detected cancers. All statistical analyses will be performed using STATA version 16 (College Station, TX: StataCorp LP).

Sample size

The sample size was determined by the primary outcome (CIN2+). In Denmark, approximately 167,000 Danish women are currently aged 65-69 years, 37,000 of whom (22,2%) reside in the CDR (intervention group) [21]. We anticipate that an estimated 55% of these will not have a record of a cervical cytology sample in the DPDB within the past five years. As follows, the study cohort will consist of a total of 91,850 women, including approximately 20,000 women in the intervention group. We assume that 50% of the eligible women in the intervention group will accept the screening offer and that the proportion of CIN2+ is 0.3% among participants. Thus, by including 10,000 women in the intervention group, the study will have a

power of 80% to detect a difference in the CIN2+ proportion of 0.1 percentage points between the intervention and reference group. The proportion of CIN2+ that was chosen (0.3%) is a conservative estimate inspired by Swedish data reporting a CIN2+ proportion of 0.38% among 56-60-year-old women attending HPV-based screening using the Cobas 4800 test [50].

Timeline

The study enrollment is expected to continue until 10,000 participants have been included in the intervention group. Invitations will be sent out prospectively over an expected 4.5 year-period starting April 2019.

PATIENT AND PUBLIC INVOLVEMENT

The research questions were developed in response to the on-going public and scientific discussion in Denmark regarding expanding the upper screening age in the organized cervical cancer screening program. No patients or patient organizations were involved in the development, design, or implementation of this study.

ETHICS AND DISSEMINATION

Ethics

According to the EU's General Data Protection Regulation, the project was listed at the record of processing activities for research projects in the CDR (j. no: 1-16-02-158-18). The study was approved by the Danish Patient Safety Authority (j.no: 3-3013-2634/1). The study protocol has been submitted to the Ethical Committee in the CDR. The Committee decided that according to the Consolidation Act on Research Ethics Review of Health Research Projects, Consolidation Act number 1083 of 15 September 2017 section 2 (1), this study is not notifiable to the Committee (j.no.: 73/2018) and informed consent is therefore not required.

Dissemination

The study protocol is registered at ClinicalTrials.gov (NCT04114968) and is made public in this protocol article. The results will be reported through publication of peer-reviewed articles in international scientific journals and presented at national and international scientific meetings. Moreover, the study results will be disseminated to healthcare stakeholders, and patient organizations at scientific meetings, and to the general public through press releases.

STRENGTHS AND LIMITATIONS OF THIS STUDY

To our knowledge, this prospective population-based intervention study will be the first to evaluate if HPV-based cervical cancer screening among older women aged 65-69 years results in an increased detection of CIN2+ cases as compared to women not invited to screening. Importantly, this study will evaluate whether the potential harms of screening in older women are outweighed by the potential benefits of decreasing the incidence of cervical (pre)-cancer [2 51]. Overall, this knowledge will address important research gaps and may help guide future screening recommendations. Compared with previous studies which report, by necessity, only the effect of cytology screening at older ages [7 22-25] it is of great value for future decision making that this study will be able to determine the effect of screening at older ages in women who have had an exit HPV-test [51]. A key strength is that the effect of the screening intervention will be measured prospectively within an organized program. From an implementation point of view, this will provide reliable estimates of the expected participation rates if extending the upper screening age together with the possibility of self-sampling would become routine. We will identify outcomes from the nationwide DPDB which has highly valid records on all pathology specimens in Denmark [33], and the selection of study participants is population-based and determined by data from the invitation module; thus eliminating both information bias and selection problems. Important limitations should be mentioned. The lack of randomization gives rise to confounding of both known and unknown risk factors. Age [6], screening history [6], comorbidities [52], education level [6], marital status [6], smoking status [7], and sexual behavior [6] may be potential confounding factors for the association between screening status and cervical (pre)-cancer development. Except for smoking status and sexual behavior, we will be able to assess whether the distribution of the remaining factors is well-balanced between the groups by using individual-level registry data [37 33 53]. Ideally, eligible women in all Danish regions should have been individually randomized to the intervention and reference group instead of being allocated to the groups based on their geographical location. Unfortunately, this was not feasible from an organizational point of view. Potentially, there may have been regional differences in the proportion of CIN2+ cases detected prior to the start of our study. If that is the

case, it may affect the impact of the intervention on CIN2+ detection rates. Fortunately, we will be able to take into account these potential regional differences by using data from the nationwide DPDB.

Detection of invasive and advanced cervical cancer is the optimal outcome measure to evaluate the effect of screening at older ages [51], but given the relative rarity of cervical cancer in older women, the length of follow-up needed and the large sample size required, we chose CIN2+ and CIN3+ as the primary and secondary outcome, respectively. Yet, it should be noted that the majority of CIN2 and CIN3 lesions detected after age 65 might not have sufficient time to progress to invasive cancer in the remaining lifespan [2]. For screening purposes, including CIN2+ and CIN3 cases as the primary and secondary outcomes, respectively, may be justified by them being treatable endpoints (conization) in older non-reproductive women according to Danish guidelines [47], while still recognizing that the detection and treatment of CIN3, especially CIN2, may be considered as overtreatment.

Trial status

Ongoing.

Acknowledgements:

Not applicable.

Contributors:

MT is the principal investigator of the study and is responsible for conducting the study overall. BA and MT conceived the original idea. Subsequently, LKP, ME, AH, MBH and JB also contributed to the design of the study. JSJ especially contributed with comments on the laboratory part of the protocol, while LKP, JB, and AH have provided clinical advice on follow-up of women with abnormal results. MT is the first author and drafted the first version of this protocol article, which was subsequently further developed by all authors, who also reviewed and approved the final version.

Funding:

The initiative and the study was partly funded by the Department of Public Health Programmes, Randers Regional Hospital, which is located in the Central Denmark Region. Some public funding had been provided by the Health Foundation (grant no.:18-B-0125) and other fundraising is on-going. The Health foundation had no role in the design of the study and collection, analysis, and interpretation of the data, and in writing the manuscript.

Competing interests:

Roche sponsors the Cobas HPV-DNA test kits and CINtec Plus test kits for the study. According to the contract between Roche and the Department of Public Health Programmes, Randers Regional Hospital, Roche has commented on the protocol article, but had no influence on the scientific process and no editorial rights pertaining to this manuscript. The authors retained the right to submit the manuscript. MT,

JB, JSJ and BA have participated in other studies with HPV test kits sponsored by Roche and self-sampling devices sponsored by Axlab. MT has received honoraria from Roche Diagnostics and AstraZeneca for lectures on HPV self-sampling and HPV triage-methods, respectively. AH has received lecture fees from AstraZeneca. All authors declare no conflicts of interest.

Patient consent:

Not required

Ethics approval:

The study protocol has been submitted to the Ethical Committee in the CDR which deemed that the study was not notifiable to the Committee and informed consent is therefore not required.

Provenance and peer review:

Not commissioned; externally peer-reviewed.

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Table 1: Overview of outcomes and planned comparisons

Outcome	Comparisons
CIN2+	Intervention vs. reference group
CIN3+	Intervention vs. reference group
Screening participation	Intervention group: self-sampling vs GP-sampling
Screening history	Intervention group: self-sampling vs GP-sampling
HPV positivity rate	Intervention group: self-sampling vs GP-sampling
HPV type distribution	Intervention group: self-sampling vs GP-sampling
Cytology results*	Intervention group
Compliance to follow-up among HPV positive self-samplers	Intervention group
Proportion and results of cervical cytology samples	Reference group
Colposcopies and conizations	Intervention vs. reference group
Cervical cancer incidence	Intervention vs. reference group

Table notes: GP: General Practitioner. CIN2+: CIN2, CIN3/AIS, and cancer. CIN3+: CIN3/AIS, and cancer.
*)Only available for women with a HPV positive GP-sample or GP-triage sample following a HPV positive self-sample.

Table 2: Overview over data sources and information

Data sources	Information
Danish Pathology Data Bank [33]	Participation (yes/no) Participation by self-sampling or GP-based screening Cervical cytology samples and results in references regions Results of self-samples, cervical cytology samples and cervical biopsies Cervical biopsy performed (yes/no) Conization performed (yes/no) Screening history
Danish Civil Registration System [35]	Residence Date of death, emigration and immigration
Danish National Patient Register [37]	Total hysterectomy and cervical amputation procedures Comorbidities
Danish Cancer Registry [54]	Cervical cancer incidence
Statistics Denmark [53]	Sociodemographic factors (e.g. age, marital status and education level)

Table notes: GP: General Practitioner.

Figure legends:

Figure 1: Map of the intervention and reference regions
Figure 2: Clinical management of women attending screening at a GP
Figure: 3 Clinical management of women attending self-sampling

Figure 1:

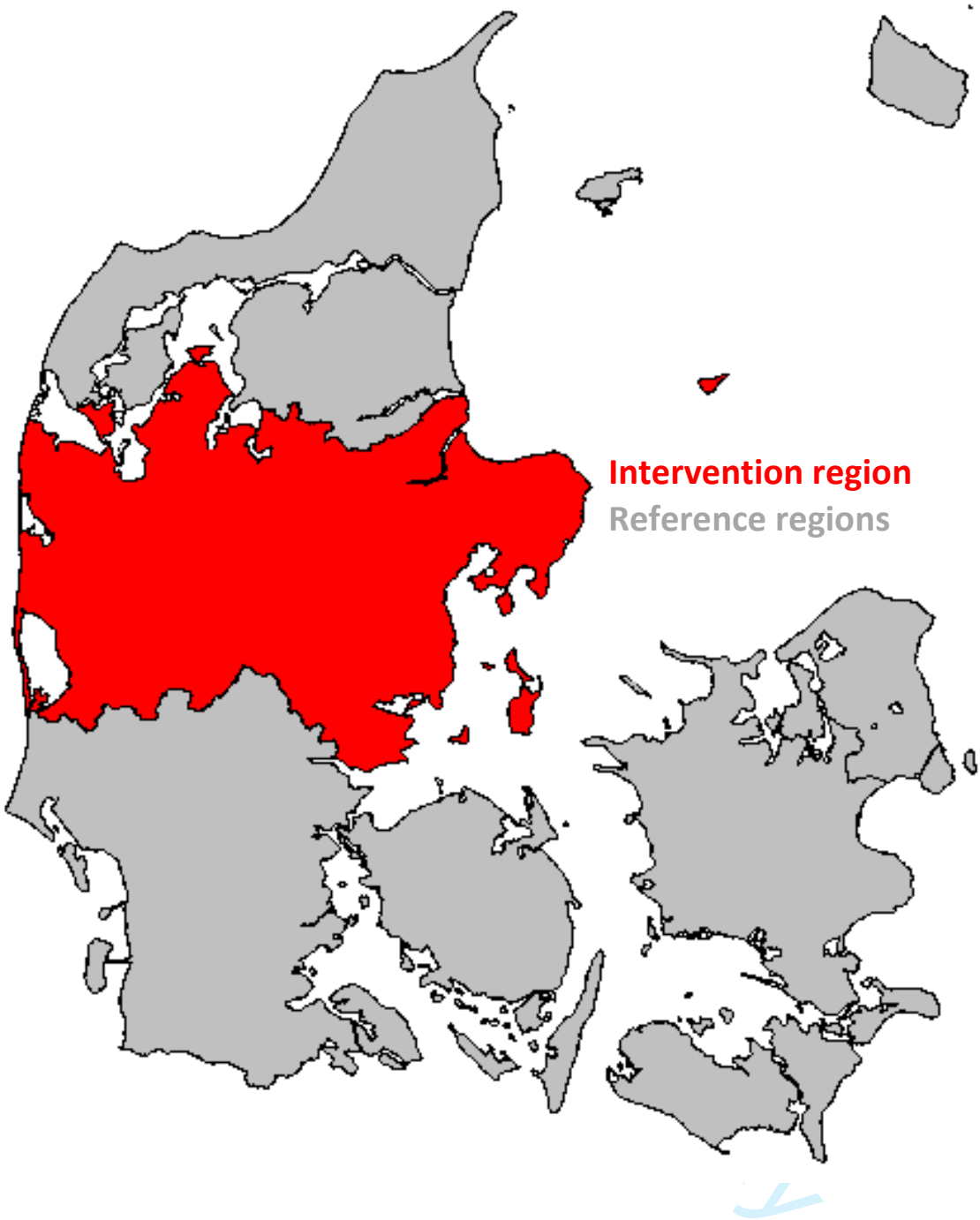


Figure 2:

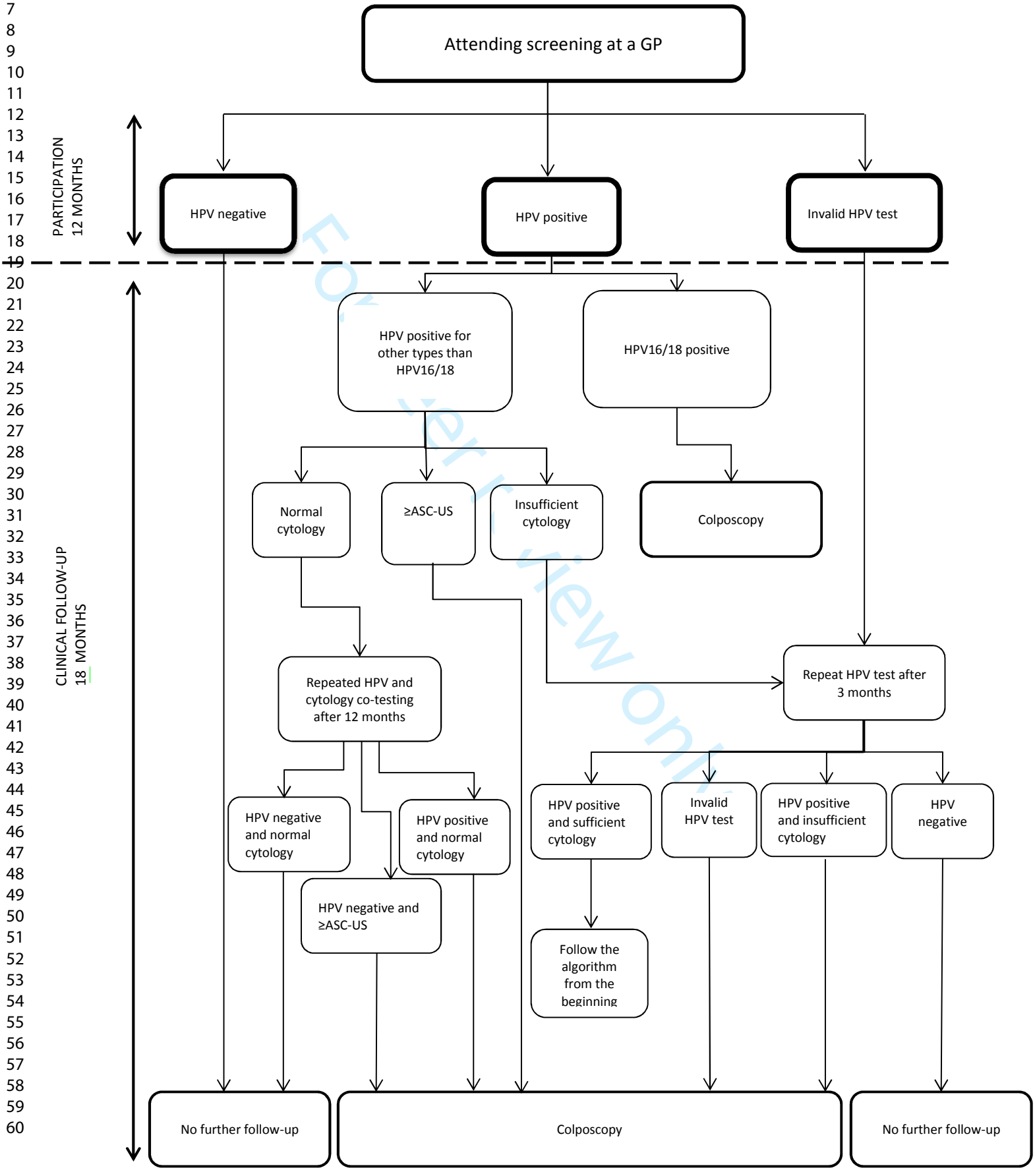
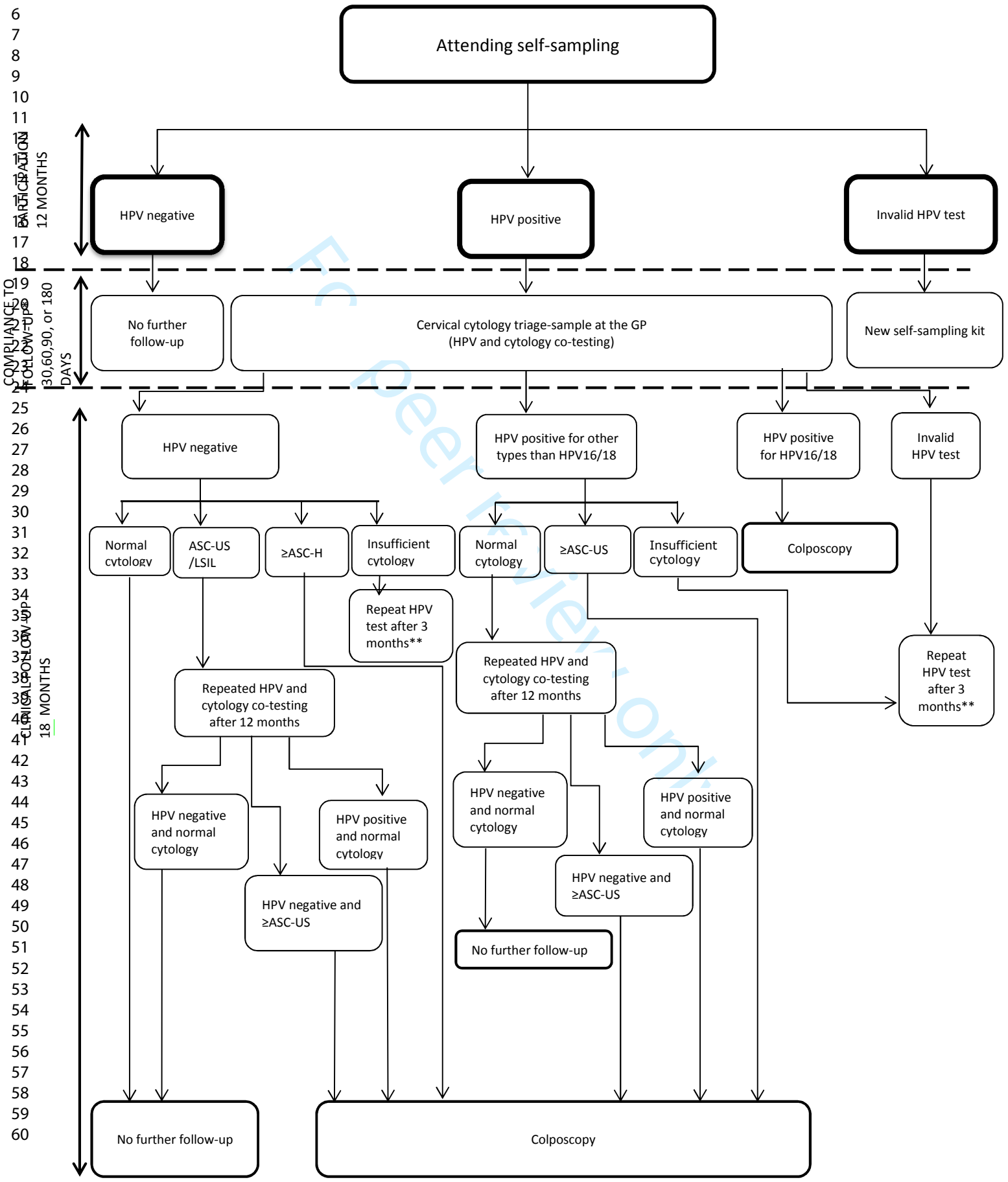


Figure notes: GP: General Practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US includes: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H); High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC) and malignant tumor cells.

Figure 3:



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BMJ Open

EXPANDING THE UPPER AGE LIMIT FOR CERVICAL CANCER SCREENING: A PROTOCOL FOR A NATIONWIDE NON-RANDOMIZED INTERVENTION STUDY

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-039636.R2
Article Type:	Protocol
Date Submitted by the Author:	09-Oct-2020
Complete List of Authors:	tranberg, mette; Regional Hospital Randers, Department of Public Health Programmes Petersen, Lone; Odense University Hospital, Department of Obstetrics and Gynecology; University of Southern Denmark, OPEN, Department of Clinical Medicine Elfström, Klara; Karolinska Institutet, Dept of Laboratory Medicine; Regional Cancer Center, Cancer Screening Unit Hammer, Anne ; Aarhus University, Department of Clinical Medicine; Regionshospitalet Herning, Department of Obstetrics and Gynecology Blaakær, Jan; University of Southern Denmark, OPEN, Department of Clinical Medicine; Odense University Hospital, Department of Obstetrics and Gynecology Bennetsen, Mary; Regional Hospital Randers, Department of Pathology Jensen, Jørgen; Statens Serum Institut, Mycoplasma Laboratory Andersen, Berit; Randers Regional Hospital, Public Helath Programs; Aarhus University, Department of Clinical Medicine
Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology, Obstetrics and gynaecology
Keywords:	EPIDEMIOLOGY, Cytopathology < PATHOLOGY, PREVENTIVE MEDICINE, PUBLIC HEALTH, GYNAECOLOGY

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EXPANDING THE UPPER AGE LIMIT FOR CERVICAL CANCER SCREENING: A PROTOCOL FOR A NATIONWIDE NON-RANDOMIZED INTERVENTION STUDY

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Number of words in text: 4,121

Number of words in abstract: 324

Number of figures: 3

Number of tables: 2

Number of references: 57

Trial registration: NCT04114968.

Keywords: Mass screening, HPV DNA testing, older women, cervical cancer screening, cervical cancer

ABSTRACT

Introduction

Cervical cancer screening ceases between the ages of 60 and 65 in most countries. Yet, a relatively high proportion of cervical cancers are diagnosed in women above the screening age. This study will evaluate if screening of women aged 65-69 years may result in increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to women not invited to screening. Invited women may choose between general practitioner (GP)-based screening or cervico-vaginal self-sampling. Furthermore, the study will assess if self-sampling is superior to GP-based screening in reaching long-term unscreened women.

Methods and Analysis

This population-based non-randomized intervention study will include 10,000 women aged 65-69 years, with no record of a cervical cytology sample or screening invitation in the 5 years before inclusion. Women who have opted-out of the screening program or have a record of hysterectomy or cervical amputation are excluded. Women residing in the Central Denmark Region are allocated to the intervention group, while women residing in the remaining four Danish regions are allocated to the reference group.

The intervention group is invited for human papillomavirus (HPV)-based screening by attending routine screening at the GP or by requesting a self-sampling kit. The reference group receives standard care which is the opportunity to have a cervical cytology sample obtained at the GP or by a gynaecologist. The study started in April 2019 and will run over the next 4.5 years.

The primary outcome will be the proportion of CIN2+ detected in the intervention and reference groups. In the intervention group, the proportion of long-term unscreened women attending GP-based screening or self-sampling will be compared.

Ethics and dissemination

The study has been submitted to the Ethical Committee in the Central Denmark Region which deemed that the study was not notifiable to the Committee and informed consent is therefore not required. The study is

approved by the Danish Data Protection Regulation and the Danish Patient Safety Authority. Results will be disseminated in peer-reviewed journals.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This population-based intervention study is the first to evaluate if expanding the upper screening age to include women aged 65-69-year and inviting them to choose between GP-based screening or self-sampling will result in increased detection of CIN2+ compared to existing practice (i.e. no screening).
- This study is the first to evaluate if self-sampling is superior to GP-based screening in reaching long-term unscreened women aged 65-69 years
- The risk of information bias and selection problems are minimized by using high-quality Danish registries and a population-based design
- The study design entails a risk of confounding due to the lack of randomization

INTRODUCTION

Cervical cancer screening ceases between the ages of 60 and 65 in most Western countries [1-3]. There is no solid evidence about which age and with which criteria to cease screening [1 4-6], but the cessation of screening in older women is often justified by a low prevalence of high-risk human papillomavirus (HPV) in women ≥55 years [7 8] and by a concern that the harms of continuing screening may outweigh potential benefits [2]. Many countries with long-established screening programs, including Denmark, experience a second incidence peak of cervical cancer around the age of 75-80 years [9 10], with a hysterectomy-corrected incidence rate of 29.4 per 100,000 person-years in women aged 75-79 [11]. These older women are more often diagnosed with advanced-stage disease, and mortality due to cervical cancer is high as compared to younger women [12 13]. It has been hypothesized that the incidence peak at older ages could be a result of a mid-life change of sexual partners or reactivation of a latent HPV infection as the immune system weakens with age [14-17]. However, a recent Danish study of HPV DNA prevalence in women aged 69 and above showed no increase in prevalence that could explain the cervical cancer peak at older ages [18]. The authors stated that this result may be explained by the fact that an HPV infection may become undetectable at a late stage in the oncogenic process [18 19]. It has also been hypothesized that the current peak in older ages could be attributed to an insufficient screening history in older birth cohorts [20]. Whatever the reason, the increasing female life expectancy (at age 65 years it is about 20 additional years) has raised the question if the upper age limit for screening should be extended to 69 or 70 years [10 21 22]. Case-control studies have reported benefits of cervical cytology screening at older ages with respect to reduced incidence and mortality [7 23-26], even among previously screened women [4]. However, a prospective evaluation of HPV-screening at ages 65-69 in a population-based intervention study including a reference group is missing. The effectiveness of cervical cancer screening among older women will depend on the participation rate and, in particular, the ability to reach long-term unscreened women, as these women have a pronounced risk of cancer [6 27]. Currently, participation in routine screening decreases with increasing age leaving a relatively high proportion of older women under-screened [10]. A potential solution to this challenge could be

to offer older women a self-sampling kit for HPV testing (self-sampling). Self-sampling is an accurate and well-accepted screening method, proven superior to physician-based screening in reaching long-term unscreened women [28 29]. Yet, it remains unknown whether an older screening population will benefit from a self-sampling offer.

OBJECTIVES

This study will evaluate if expanding the upper screening age to include women aged 65-69-year and inviting them to choose between general practitioner (GP)-based screening or self-sampling results in increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to existing practice where women in this age group are not invited to routine screening. Furthermore, it will be assessed whether self-sampling is better than GP-based screening in reaching long-term unscreened women.

HYPOTHESES

We hypothesize that expanding the upper screening age will result in increased detection of CIN2+ cases and, long-term, potentially reduce the cervical cancer incidence compared to women not invited to screening. Finally, we hypothesize that self-sampling will be superior to GP-based screening in reaching long-term unscreened women.

METHODS AND ANALYSIS

Setting

Organized cervical cancer screening was introduced in parts of Denmark in the 1960s and became nationwide in the late 1990s [30 31]. Screening in Denmark is currently organized by the five regions (North, Central, South, Zealand, and Capital) following national guidelines [31 32]. Cervical cancer screening is centralized to one or a few pathology departments in each region [31]. Danish women are invited to schedule an appointment with their GP for liquid-based cytology screening every third year when aged 23 to 49 years and every fifth year when aged 50 to 64 years[31]. Since 2012, women aged 60-64 years have been screened with an HPV DNA-check-out test, after which HPV negative women can exit the program without consideration of their previous screening history [32]. Outside the organized program, women can have a cervical cytology sample taken by a GP or a gynecologist opportunistically or due to clinical symptoms at any time. In Denmark, cervical cancer screening, including clinical follow-up and treatment, is free of charge [31].

The intervention in this study will be run by the Department of Public Health Programmes, Randers Regional Hospital in the Central Denmark Region (CDR). The CDR is the second largest region in Denmark covering approximately one-fourth of the Danish population (1.2 million inhabitants)[22]. In the CDR, the Department of Public Health Programmes oversees sending screening invitations, reminders, and test results, while the Department of Pathology, Randers Regional Hospital handles and analyses all cervical cytology samples.

The call-recall invitation system

The Danish screening program is based on an integrated call-recall system using data from the invitation module in the nationwide Danish Pathology Databank (DPDB) [31 33 34]. The Conseillers en Gestion et Informatique (CGI) Institute operates the call-recall system and it is designed so that each region only invites women residing in their catchment area. The call-recall system invites women for screening after the age-specific interval has passed since their latest invitation or cervical cytology sample (whichever came last). Samples obtained opportunistically, symptomatically or as part of surveillance are also recorded in the DPDB and postpone the next invitation. The system also keeps track of women who are ineligible for screening because they have actively opted out of the program or have had a hysterectomy. The latter registration is rather incomplete and varies between the regions. In detail, the invitation module links cervical cytology data (Systematized Nomenclature of Medicine, SNOMED codes: T8X3* and T8X210) from the DPDB's main pathology module with information about residency and vital status from the Danish Civil Registration System [35 36]. Linkage is performed using the unique Civil Personal Registration number (CPR), which is assigned to every Danish citizen upon birth and to residents upon immigration [36]. The CPR number is used by all citizens for any contact to the Danish health care system.

Design and eligibility criteria

This study is a nationwide prospective population-based non-randomized intervention study (i.e. a quasi-experimental design) [37]. Women will consecutively be deemed eligible if they meet the following criteria at the time of inclusion: aged 65 to 69 years; resident in Denmark for the past 5 years; no record of a cervical cytology sample or invitation in the past 5 years; not registered in the invitation module as having actively opted out of the screening program or having a record of total hysterectomy or cervical amputation in the Danish National Patient Register [38]. Eligible women residing in the CDR will be allocated to the intervention group, while women residing in the other four Danish regions will be allocated to the reference group (Figure 1). In the intervention group, the invitation module will be set-up to identify women fitting the

inclusion criteria, and simultaneously a comparable list of eligible women in the reference group will be compiled by CGI at the Department of Public Health Programmes' request. Inclusion started in April 2019 and eligible women will be identified with six months' intervals until the desired number of women have been included. The follow-up period for the included women will start on the date of the invitation and end at time of death, emigration, cervical amputation, total hysterectomy, or end of study. Women that move between the intervention region and reference regions in the follow-up period will subsequently be excluded from the analysis.

Intervention group

Women living in the CDR and therefore eligible for the intervention group will be invited to HPV-based cervical cancer screening by either scheduling an appointment for having a cervical cytology sample collected at their GP or collecting a cervico-vaginal sample themselves in their own home using a self-sampling kit. Women will receive an invitation and an information sheet by digital mail, while those exempted from digital mail as per routine will receive the information by postal mail [39]. The invitation explains how to request the self-sampling kit and states that once the woman attends screening it will implicitly represent her consent to store her sample for future quality improvement of the screening program. A phone number for calling the study investigator to decline this option will be available. Test results, including follow-up recommendations, will be sent to the women by digital or postal mail and the woman's GP will receive an electronic copy of the test result. Around 98% of all residents in Denmark are listed with a GP [40]. As per routine, non-participants will receive up to two reminders at 3 and 6 months post invitation [31]. All information will be in Danish.

The self-sampling kit

The self-sampling kit can be requested by phone or through a study webpage. After receiving the orders in the department, the kit will be mailed to the women within four business days. The kit includes the dry Evalyn® brush self-sampling device (Rovers Medical Devices B.V, Oss, Netherlands) [41], written and picture-

based user instructions on how to collect and mail the self-sample, and a pre-stamped return envelope addressed to the Department of Pathology, Randers Regional Hospital [42]. The acceptability of both the self-sampling device and user instructions has been carefully evaluated in previous studies, although among younger women (30-59 years) [43 44].

Processing and analysis of samples

In the intervention group, all samples will be prepared, processed and analyzed at the Department of Pathology, Randers Regional Hospital according to the routine laboratory protocols. All HPV testing will be performed using the clinically validated and Federal Drug Agency (FDA)-approved Cobas® 4800 DNA test (Roche Diagnostics, Switzerland) [45], as this is the routine test platform used in the CDR. The test is an automated real-time PCR-based test designed to detect high-risk HPV types: 16,18,31,33,35,39,45,51,52, 56,58,59,66 and 68 [45 46] and is validated for use on SurePath collected samples [47]. Results will be reported as 1) HPV negative, 2) HPV positive (HPV16, HPV18 and/ or other HPV types) or 3) invalid [42]. All samples with an invalid test result will be re-tested, and the second result will be considered definitive. The Cobas test measures beta-globin as an internal control for sample cellularity, valid sample extraction, and amplification [46].

As per routine, cervical cytology samples taken by the GPs will be collected using a cervical brush and rinsed in 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and mailed to the Department of Pathology, Randers Regional Hospital for processing and HPV testing. For HPV positive women, reflex cytology testing will be performed on the residual cellular Surepath material. Cytology will be interpreted by cytotechnologists using computer-assisted microscopy and categorized per the Bethesda 2014 grading system as normal; inadequate; Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H). High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC), and malignant tumor cells. At the laboratory, the

Evalyn brush device will be rinsed into 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and processed as previously described [43].

From the cervical cytology samples and self-samples, 2 mL of the SurePath medium will be placed in test tubes for HPV testing. The residual eluate material from these samples will be stored at -80˚C for future extended genotyping and DNA methylation analysis. As part of the study, and only in the intervention group, p16/Ki67 cytology dual staining (CINtec® PLUS cytology kit, Roche Diagnostics, Switzerland) will be performed consecutively on the residual SurePath cell-pellet obtained from women with an HPV positive cervical cytology sample and sufficient material for cytology testing. The dual staining result will not affect the clinical management of the woman. Except for the dual staining result, all test results will routinely be registered in the DPDB [34].

Clinical management

Figures 2 and 3 show the recommended, and therefore, expected clinical management for women in the intervention group, but management may deviate depending on the clinical presentation of the individual woman. The recommendations are in accordance with the routine screening guidelines for 60-64-year-old women and the new guidelines for clinical management of older women with dysplasia and HPV [32 48].

Women who are positive for HPV16 or 18 AND other types will be managed similar to HPV16/18 positive women.

For women attending GP-based screening, those who tested HPV negative will have no further follow-up (Figure 2). Women tested positive for HPV 16/18 will be referred directly to colposcopy (regardless of the cytology result). Women tested HPV positive for other types than HPV16/18 with ASC-US or more severe cytological abnormalities will be referred to colposcopy, while women with HPV types other than HPV16/18 and normal cytology will undergo repeated co-testing (HPV and cytology) after 12 months and will be referred for colposcopy if either test result is positive.

Figure 3 presents the follow-up recommendations for women attending self-sampling. Women who tested HPV negative in their self-sample will have no further follow-up. Women with an HPV positive self-sample

(any genotype) will be advised to have a cervical cytology triage sample taken by their GP within 30 days to evaluate the need for referral to colposcopy. This triage sample will be co-tested with HPV and cytology. Women tested HPV negative with normal cytology will have no further follow-up, while those with ASC-US/LSIL cytology will undergo a repeat co-test (HPV and cytology) after 12 months and will be referred for colposcopy if either test result is positive. Those with ASC-H or more severe abnormalities will be referred for colposcopy. Women with an HPV positive triage cytology sample will follow the same recommendation as described for the GP-based screening (Figure 2).

For women referred for colposcopy, cervical punch biopsies will be taken from suspicious areas, supplemented with random biopsies according to Danish guidelines [49]. Some women may also undergo a diagnostic conization as part of a clinical "see-and-treat" study [50]. Histological examination of the cervical biopsies will be carried out at different local Pathology Departments and graded using the Cervical Intraepithelial Neoplasia (CIN) classification as normal (including inflammation and non-specific reactive features), CIN (not specified), CIN grade 1, 2 or 3/AIS, or invasive cancer.

Reference group

Women in the reference group will receive usual care which, for 65-69-year-old women, is the opportunity to have a cervical cytology sample obtained at their GP or by a gynecologist for whatever reason. The women will not receive a screening invitation, but will be assigned individual pseudo screening invitation dates allowing comparison between the groups in our statistical analysis. In the following, the term "invitation date" will be used for both for the "true invitation dates" in the intervention group and "pseudo invitation dates" in the reference group. In all reference regions, samples from this age group are expected to be tested for HPV. Differences across the four regions are found in the HPV assay [18] and there may be minor differences in the triage-strategies, which may result in differences in indication for colposcopy referral. However, clinical management of women referred for colposcopy is expected to follow national guidelines as described above [48 49].

Outcomes

Overview of outcomes and planned comparisons is seen in Table 1.

In both groups, the primary outcome will be the proportion of CIN2+ (CIN2, CIN3/AIS, and cancer) detected within 18 months following registration of a cervical cytology sample or self-sample. The proportion of CIN3+ (CIN3/AIS, and cancer) will be a secondary outcome. The most severe histological test result will be used if more than one result is available in the follow-up period. Other outcomes in the intervention group will be screening participation measured 12 months after the invitation date, defined by returning a self-sample or having a cervical cytology taken; screening history of participants, stratified by sampling procedure; HPV positivity rate and HPV type distribution in self-samples versus GP-collected cervical cytology samples; cytology results, and the percentage of HPV positive self-samplers undergoing appropriate follow-up. Compliance to follow-up after self-sampling will be defined as attending a GP for a cervical cytology-triage sample within 30, 60, 90 or 180 days after mailing of the test results. The proportion and results of cervical cytology samples obtained among women not invited for screening will be identified in the reference group and measured 12 months post invitation date. As in another study [2], the primary measure of harms will in both groups be the number of colposcopies/conizations performed, both overall and relatively to ≤CIN1, CIN2, CIN3/AIS, and cancers detected within a follow-up period of 18 months after registration of a cervical cytology sample or self-sample. Long-term outcomes will be cervical cancer incidence rates reported by groups at 5- and 10-years post invitation dates. A description of histological type and FIGO stage of the detected cervical cancers will be provided.

Data sources and statistical analysis

An overview of data sources and information is seen in Table 2.

Baseline characteristics in both groups will be presented using descriptive statistics (numbers and proportions) on screening history, comorbidities, sociodemographic factors (e.g. age, marital status, and education level). Screening history will be categorized based on the woman's screening history in a 15-year period before screening exit (i.e. age 50-64) according to the results of the cytology screening at age 50-59

and the HPV-exit test at age 60-64. The categorization of screening history is expected to be as follows [6]: 1) "Sufficiently screened with normal results" if women had a) at least one normal cytology at age 50-54 and b) at least one normal cytology at age 55-59, and c) no abnormal cytology (ASC-US or worse) at age 50-59, and d) HPV negative at age 60-64; 2) "Insufficiently screened with normal results" if women had one or more cytology samples with only normal results, but only in one or two age categories (50-54, 55-59 or 60-64); 3) "Long-term unscreened" if no cervical cytology sample at age 50-64; and 4) "Abnormal screening" if women a) had ASC-US or worse at least once at age 50-59 or b) HPV positive at age 60-64.

Screening participation, cervical cytology samples, numbers of colposcopies/conizations performed, compliance to follow-up among positive self-samplers, and disease outcomes (HPV positivity rate and histological outcomes) will be estimated as proportions. Participation in the intervention group will be reported by age groups, screening history, and sampling method (GP versus self-sampling). Regression analyses will be used to estimate the association between CIN2+ detection in women offered cervical cancer screening compared to those not offered screening. Both crude and adjusted estimates will be presented with 95% confidence intervals (CIs). Cumulative incidence rates of cervical cancer among women in the intervention and reference groups will be reported, including the distribution of the histological types and FIGO stage of the detected cancers. All statistical analyses will be performed using STATA version 16 (College Station, TX: StataCorp LP).

Sample size

The sample size was determined by the primary outcome (CIN2+). In Denmark, approximately 167,000 Danish women are currently aged 65-69 years, 37,000 of whom (22,2%) reside in the CDR (intervention group) [22]. We anticipate that an estimated 55% of these will not have a record of a cervical cytology sample in the DPDB within the past five years. As follows, the study cohort will consist of a total of 91,850 women, including approximately 20,000 women in the intervention group. We assume that 50% of the eligible women in the intervention group will accept the screening offer and that the proportion of CIN2+ is 0.3% among participants. Thus, by including 10,000 women in the intervention group, the study will have a

power of 80% to detect a difference in the CIN2+ proportion of 0.1 percentage points between the intervention and reference group. The proportion of CIN2+ that was chosen (0.3%) is a conservative estimate inspired by Swedish data reporting a CIN2+ proportion of 0.38% among 56-60-year-old women attending HPV-based screening using the Cobas 4800 test [51].

Timeline

The study enrollment is expected to continue until 10,000 participants have been included in the intervention group. Invitations will be sent out prospectively over an expected 4.5 year-period starting April 2019.

PATIENT AND PUBLIC INVOLVEMENT

The research questions were developed in response to the on-going public and scientific discussion in Denmark regarding expanding the upper screening age in the organized cervical cancer screening program. No patients or patient organizations were involved in the development, design, or implementation of this study.

ETHICS AND DISSEMINATION

Ethics

According to the EU's General Data Protection Regulation, the project was listed at the record of processing activities for research projects in the CDR (j. no: 1-16-02-158-18). The study was approved by the Danish Patient Safety Authority (j.no: 3-3013-2634/1). The study protocol has been submitted to the Ethical Committee in the CDR. The Committee decided that according to the Consolidation Act on Research Ethics Review of Health Research Projects, Consolidation Act number 1083 of 15 September 2017 section 2 (1), this study is not notifiable to the Committee (j.no.: 73/2018) and informed consent is therefore not required.

Dissemination

The study protocol is registered at ClinicalTrials.gov (NCT04114968) and is made public in this protocol article. The results will be reported through publication of peer-reviewed articles in international scientific journals and presented at national and international scientific meetings. Moreover, the study results will be disseminated to healthcare stakeholders, and patient organizations at scientific meetings, and to the general public through press releases.

STRENGTHS AND LIMITATIONS OF THIS STUDY

To our knowledge, this prospective population-based intervention study will be the first to evaluate if HPV-based cervical cancer screening among older women aged 65-69 years results in an increased detection of CIN2+ cases as compared to women not invited to screening. Importantly, this study will evaluate whether the potential harms of screening in older women are outweighed by the potential benefits of decreasing the incidence of cervical (pre)-cancer [2 52]. Overall, this knowledge will address important research gaps and may help guide future screening recommendations. Compared with previous studies which report, by necessity, only the effect of cytology screening at older ages [7 23-26] it is of great value for future decision making that this study will be able to determine the effect of screening at older ages in women who have had an exit HPV-test [52].

A key strength is that the effect of the screening intervention will be measured prospectively within an organized program. From an implementation point of view, this will provide reliable estimates of the expected participation rates if extending the upper screening age together with the possibility of self-sampling would become routine. We will identify outcomes from the nationwide DPDB which has highly valid records on all pathology specimens in Denmark [34], and the selection of study participants is population-based and determined by data from the invitation module; thus eliminating both information bias and selection problems. Important limitations should be mentioned. The lack of randomization gives rise to confounding of both known and unknown risk factors. Age [6], screening history [6], comorbidities [53], education level [6], marital status [6], smoking status [7], and sexual behavior [6] may be potential confounding factors for the association between screening status and cervical (pre)-cancer development. Except for smoking status and sexual behavior, we will be able to assess whether the distribution of the remaining factors is well-balanced between the groups by using individual-level registry data [38 34 54]. Ideally, eligible women in all Danish regions should have been individually randomized to the intervention and reference group instead of being allocated to the groups based on their geographical location. Unfortunately, this was not feasible from an organizational point of view. Potentially, there may have been regional differences in

the proportion of CIN2+ cases detected prior to the start of our study. If that is the case, it may affect the impact of the intervention on CIN2+ detection rates. Fortunately, we will be able to take into account these potential regional differences by using data from the nationwide DPDB.

Detection of invasive and advanced cervical cancer is the optimal outcome measure to evaluate the effect of screening at older ages [52], but given the relative rarity of cervical cancer in older women, the length of follow-up needed and the large sample size required, we chose CIN2+ and CIN3+ as the primary and secondary outcome, respectively. Yet, it should be noted that the majority of CIN2 and CIN3 lesions detected after age 65 might not have sufficient time to progress to invasive cancer in the remaining lifespan [2]. For screening purposes, including CIN2+ and CIN3 cases as the primary and secondary outcomes, respectively, may be justified by them being treatable endpoints (conization) in older non-reproductive women according to Danish guidelines [48], while still recognizing that the detection and treatment of CIN3, and especially CIN2, may be considered as overtreatment, because an unknown proportion of these lesions would never have progressed to cancer in the woman's lifetime[55]. Specifically, it is important to take into account that conization is associated with an increased risk of bleeding and stenosis, which may hinder or challenge sampling from the cervix post-conization[52], and that false-positive screening results may place some women in a surveillance cycle of unclear end, which may cause distress[55].

Trial status

Ongoing.

Acknowledgements:

Not applicable.

Contributors:

MT is the principal investigator of the study and is responsible for conducting the study overall. BA and MT conceived the original idea. Subsequently, LKP, ME, AH, MBH and JB also contributed to the design of the study. JSJ especially contributed with comments on the laboratory part of the protocol, while LKP, JB, and AH have provided clinical advice on follow-up of women with abnormal results. MT is the first author and drafted the first version of this protocol article, which was subsequently further developed by all authors, who also reviewed and approved the final version.

Funding:

The initiative and the study was partly funded by the Department of Public Health Programmes, Randers Regional Hospital, which is located in the Central Denmark Region. Some public funding had been provided by the Health Foundation (grant no.:18-B-0125) and other fundraising is on-going. The Health foundation had no role in the design of the study and collection, analysis, and interpretation of the data, and in writing the manuscript.

Competing interests:

Roche sponsors the Cobas HPV-DNA test kits and CINtec Plus test kits for the study. According to the contract between Roche and the Department of Public Health Programmes, Randers Regional Hospital, Roche has commented on the protocol article, but had no influence on the scientific process and no

editorial rights pertaining to this manuscript. The authors retained the right to submit the manuscript. MT, JB, JSJ and BA have participated in other studies with HPV test kits sponsored by Roche and self-sampling devices sponsored by Axlab. MT has received honoraria from Roche Diagnostics and AstraZeneca for lectures on HPV self-sampling and HPV triage-methods, respectively. AH has received lecture fees from AstraZeneca. All authors declare no conflicts of interest.

Patient consent:

Not required

Ethics approval:

The study protocol has been submitted to the Ethical Committee in the CDR which deemed that the study was not notifiable to the Committee and informed consent is therefore not required.

Provenance and peer review:

Not commissioned; externally peer-reviewed.

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Table 1: Overview of outcomes and planned comparisons

Outcome	Comparisons
CIN2+	Intervention vs. reference group
CIN3+	Intervention vs. reference group
Screening participation	Intervention group: self-sampling vs GP-sampling
Screening history	Intervention group: self-sampling vs GP-sampling
HPV positivity rate	Intervention group: self-sampling vs GP-sampling
HPV type distribution	Intervention group: self-sampling vs GP-sampling
Cytology results*	Intervention group
Compliance to follow-up among HPV positive self-samplers	Intervention group
Proportion and results of cervical cytology samples	Reference group
Colposcopies and conizations	Intervention vs. reference group
Cervical cancer incidence	Intervention vs. reference group

Table notes: GP: General Practitioner. CIN2+: CIN2, CIN3/AIS, and cancer. CIN3+: CIN3/AIS, and cancer.
*)Only available for women with a HPV positive GP-sample or GP-triage sample following a HPV positive self-sample.

Table 2: Overview over data sources and information

Data sources	Information
Danish Pathology Data Bank [34]	Participation (yes/no)
	Participation by self-sampling or GP-based screening
	Cervical cytology samples and results in references regions
	Results of self-samples, cervical cytology samples and cervical biopsies
	Cervical biopsy performed (yes/no)
	Conization performed (yes/no)
	Screening history
Danish Civil Registration System [36]	Residence
	Date of death, emigration and immigration
Danish National Patient Register [38]	Total hysterectomy and cervical amputation procedures
	Comorbidities
Danish Cancer Registry [56]	Cervical cancer incidence
Statistics Denmark [54]	Sociodemographic factors (e.g. age, marital status and education level)

Table notes: GP: General Practitioner.

Figure legends:

Figure 1: Map of the intervention and reference regions
Figure 2: Clinical management of women attending screening at a GP
Figure: 3 Clinical management of women attending self-sampling

Figure 1:

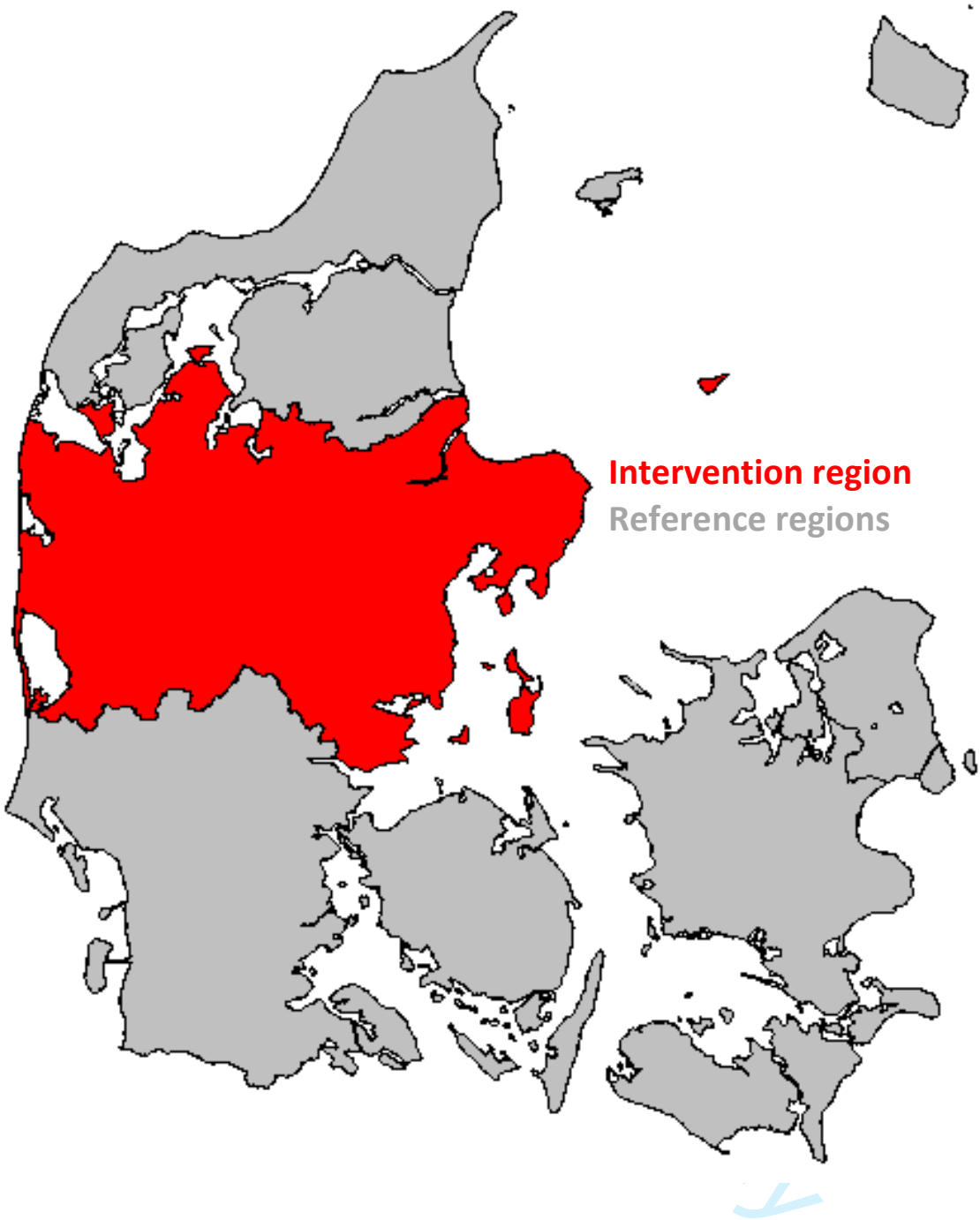


Figure 2:

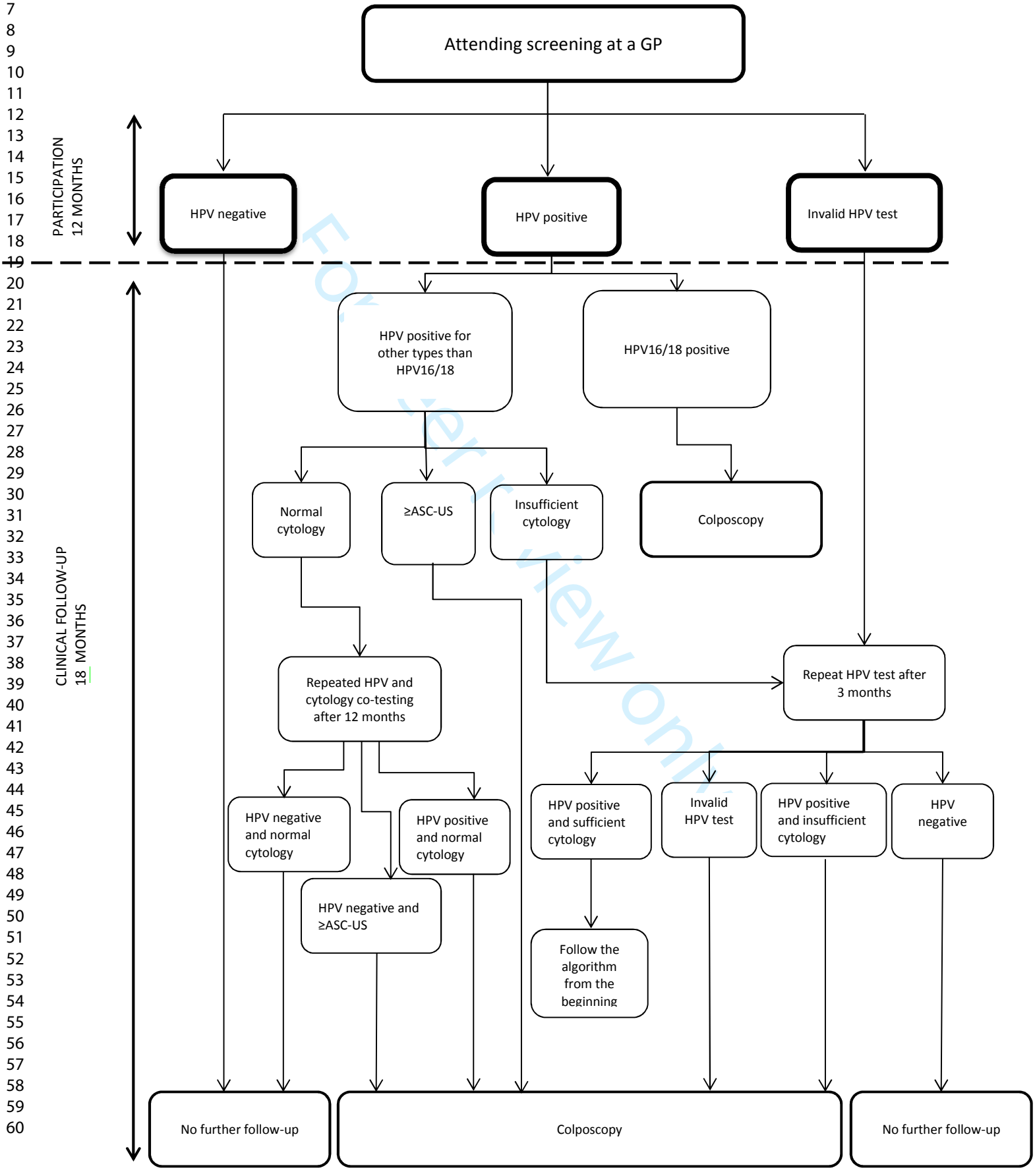
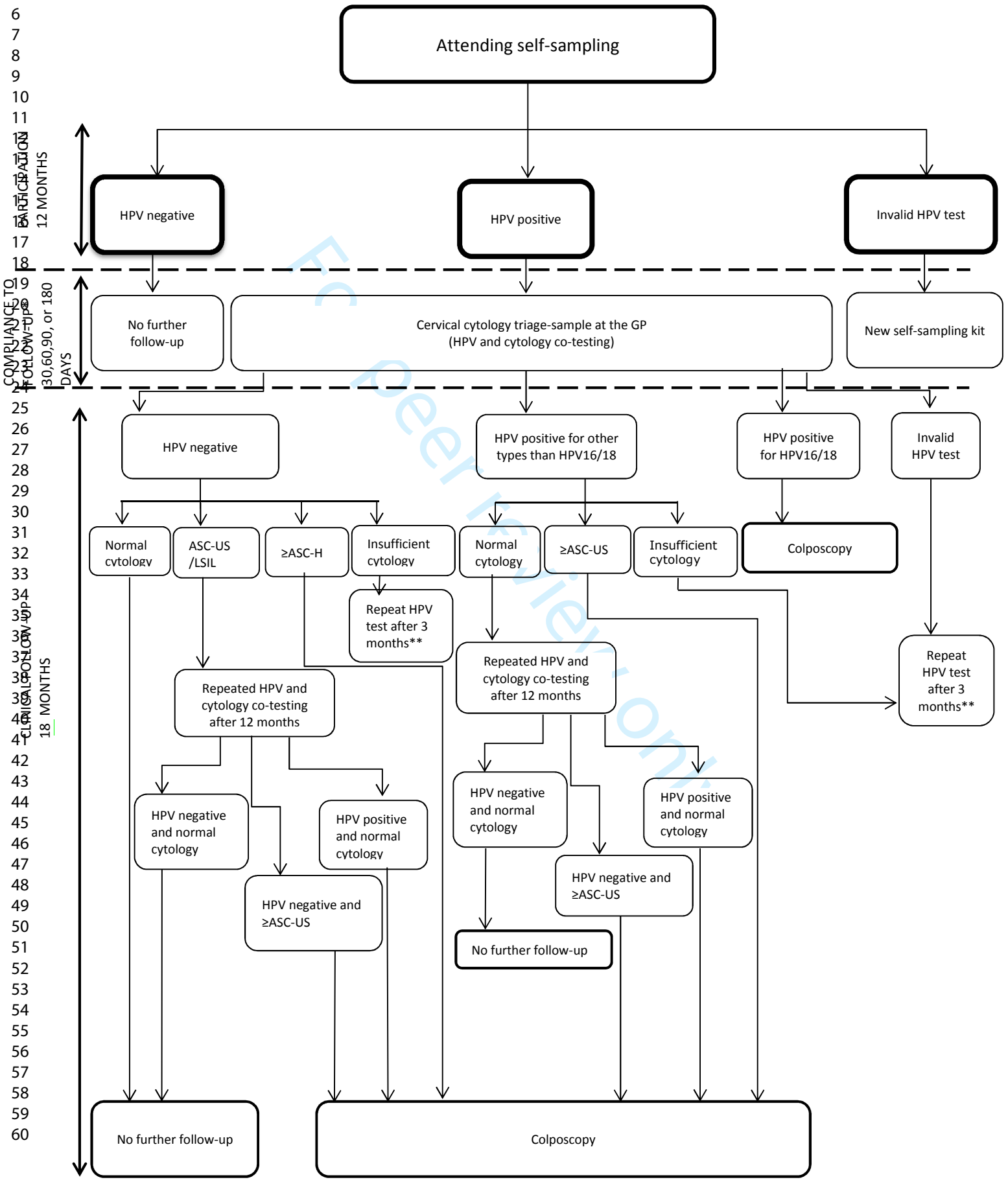


Figure notes: GP: General Practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US includes: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H); High-grade Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC), Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC) and malignant tumor cells.

Figure 3:



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