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

STUDY

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

48 **ABSTRACT**

50 **Introduction**

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

57 **Methods**

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

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

67 **Analyses**

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

69 **Ethics and dissemination**

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

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

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For peer review only

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

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85 INTRODUCTION

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87 Cervical cancer screening ceases between the ages of 60 and 65 in most Western countries (1-3). There is
88 no solid evidence about which age and with which criteria to cease screening (1, 4-6), but the cessation of
89 screening in older women is often justified by a low prevalence of high-risk human papillomavirus (HPV) in
90 women ≥ 55 years (7, 8) and by a concern that the harms of continuing screening may outweigh potential
91 benefits (2). Many countries with long-established screening programs, including Denmark, experience a
92 second incidence peak of cervical cancer around the age of 75-80 years (9, 10). These older women are
93 more often diagnosed with advanced-stage disease and mortality due to cervical cancer is high as
94 compared to younger women (11, 12). It has been hypothesized that the incidence peak could be a result
95 of a mid-life change of sexual partners or reactivation of a latent HPV infection as the immune system
96 weakens with age (13-16). However, a recent Danish study of HPV DNA prevalence in women aged 69 and
97 above showed no increase in prevalence that could explain the cervical cancer peak in this age group (17).
98 It has also been hypothesized that the current peak in older ages could be attributed to an insufficient
99 screening history in older birth cohorts (18). Whatever the reason, the increasing female life expectancy (at
100 age 65 years it is about 20 additional years) has raised the question if the upper age limit for screening
101 should be extended to 69 or 70 years (10, 19, 20). Case-control studies have reported benefits of cervical
102 cytology screening at older ages with respect to reduced incidence and mortality (7, 21-24), even among
103 previously screened women (4). However, a prospective evaluation of HPV-screening at ages 65-69 in a
104 population-based intervention study including a reference group is missing.
105 The effectiveness of cervical cancer screening among older women will depend on the participation rate
106 and, in particular, the ability to reach long-term unscreened women, as these women have a pronounced
107 risk of cancer (6, 25). Currently, participation in routine screening decreases with increasing age leaving a
108 relatively high proportion of older women under-screened (10). A potential solution to this challenge could
109 be to offer older women a self-sampling kit for HPV testing (HPV self-sampling). HPV self-sampling is an
110 accurate and well-accepted screening method, proven superior to physician-based screening in reaching

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4 111 long-term unscreened women (26, 27). Yet, it remains unknown whether an older screening population will
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6 112 benefit from a self-sampling offer.
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9 113 **OBJECTIVES**

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12 114 This study will evaluate if expanding the upper screening age to include women aged 65-69-year results in
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14 115 increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to women not
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16 116 offered screening and will establish whether HPV self-sampling is better than general practitioner (GP)-
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18 117 based screening in reaching long-term unscreened women.
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20 118 **HYPOTHESES**

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23 119 We hypothesize that expanding the upper screening age will result in increased detection of CIN2+ cases
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26 120 and, long-term, potentially reduce the cervical cancer incidence compared to women not offered screening.
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28 121 Finally, we hypothesize that HPV self-sampling will be superior to GP-based screening in reaching long-term
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30 122 unscreened women.
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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

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4 170 women fitting the inclusion criteria, and simultaneously a comparable list of eligible women in the
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6 171 reference group will be compiled by CGI at the Department of Public Health Programmes' request. Inclusion
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9 172 started in April 2019 and eligible women will be identified with six months intervals until the desired
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11 173 number of women have been included. The follow-up period for the included women will start on the date
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13 174 of the invitation and end at time of death, emigration, cervical amputation, total hysterectomy, or end of
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15 175 study. Women that move between the intervention region and reference regions in the follow-up period
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18 176 will subsequently be excluded from the analysis.

21 177 **Intervention group**

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23 178 Women living in the CDR and therefore eligible for the intervention group will be invited to HPV-based
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25 179 cervical cancer screening by either scheduling an appointment for having a cervical cytology sample
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28 180 collected at their GP or collecting a cervico-vaginal sample themselves in their own home using a self-
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30 181 sampling kit. Women will receive an invitation and an information sheet by digital mail, while those
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32 182 exempted from digital mail as per routine will receive the information by postal mail (37). The invitation
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34 183 explains how to request the self-sampling kit and states that once the woman attends screening it will
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37 184 implicitly represent her consent to store her sample for future quality improvement of the screening
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39 185 program. A phone number for calling the study investigator to decline this option will be available. Test
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41 186 results, including follow-up recommendations, will be sent to the women by digital or postal mail and the
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43 187 woman's GP will receive an electronic copy of the test result. Around 98% of all residents in Denmark are
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45 188 listed with a GP (38). As per routine, non-participants will receive up to two reminders at 3 and 6 months
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48 189 post invitation (29). All information will be in Danish.

50 190 **The self-sampling kit**

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52 191 The self-sampling kit can be requested by phone or through a study webpage. After receiving the orders in
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55 192 the department, the kit will be mailed to the women within four business days. The kit includes the dry
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57 193 Evalyn® brush self-sampling device (Rovers Medical Devices B.V, Oss, Netherlands) (39), written and
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4 194 picture-based user instructions on how to collect and mail the self-sample, and a pre-stamped return
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6 195 envelope addressed to the Department of Pathology, Randers Regional Hospital (40). The acceptability of
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9 196 both the self-sampling device and user instructions has been carefully evaluated in previous studies,
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11 197 although among younger women (30-59 years) (41, 42).

13 198 **Processing and analysis of samples**

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16 199 In the intervention group, all samples will be prepared, processed and analyzed at the Department of
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18 200 Pathology, Randers Regional Hospital according to the routine laboratory protocols. All HPV testing will be
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20 201 performed using the clinically validated and Federal Drug Agency (FDA)-approved Cobas® 4800 DNA test
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22 202 (Roche Diagnostics, Switzerland) (43), as this is the routine test platform used in the CDR. The test is an
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25 203 automated real-time PCR-based test designed to detect high-risk HPV types: 16,18,31,33,35,39,45,51,52,
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27 204 56,58,59,66 and 68 (43, 44). Results will be reported as 1) HPV negative, 2) HPV positive (HPV16, HPV18
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29 205 and/ or other HPV types) or 3) invalid (40). All samples with an invalid test result will be re-tested, and the
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31 206 second result will be considered definitive. The Cobas test measures beta-globin as an internal control for
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34 207 sample cellularity, valid sample extraction, and amplification (44).

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36 208 As per routine, cervical cytology samples taken by the GPs will be collected using a cervical brush and rinsed
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38 209 in 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and mailed to the Department of Pathology,
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40 210 Randers Regional Hospital for processing and HPV testing. For HPV positive women, reflex cytology testing
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42 211 will be performed on the residual cellular Surepath material. Cytology will be interpreted by
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45 212 cytotechnologists using computer-assisted microscopy and categorized per the Bethesda 2015 grading
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47 213 system as normal; inadequate; Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade
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49 214 Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot exclude HSIL (ASC-H). High-grade
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51 215 Squamous Intraepithelial Lesion (HSIL); Squamous Cell Carcinoma (SCC); Atypical Glandular Cells (AGC),
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54 216 Adenocarcinoma In Situ (AIS); Adenocarcinoma (ACC), and malignant tumor cells. At the laboratory, the
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56 217 Evalyn brush device will be rinsed into 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and
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58 218 processed as previously described (41).

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

226 **Clinical management**

227 Figures 2 and 3 show the recommended, and therefore, expected clinical management for women in the
228 intervention group, but management may deviate depending on the clinical presentation of the individual
229 woman. The recommendations are in accordance with the routine screening guidelines for 60-64-year-old
230 women and the new guidelines for clinical management of older women with dysplasia and HPV (30, 45).

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

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

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

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

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

254 **Reference group**

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

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266 **Outcomes**

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

283 **Data sources and statistical analysis**

284 An overview of data sources and information is seen in Table 1.
285 Baseline characteristics in both groups will be presented using descriptive statistics (numbers and
286 proportions) on screening history and sociodemographic factors (e.g. age, marital status, and education
287 level). Screening history will be categorized based on the woman's screening history in a 15-year period
288 before screening exit (i.e. age 50-64) according to the results of the cytology screening at age 50-59 and the
289 HPV-exit test at age 60-64. The categorization of screening history is expected to be as follows (6):

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4 290 1) "Sufficiently screened with normal results" if women had a) at least one normal cytology at age 50-54
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6 291 and b) at least one normal cytology at age 55-59, and c) no abnormal cytology (ASC-US or worse) at age 50-
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8 292 59, and d) HPV negative at age 60-64; 2) "Insufficiently screened with normal results" if women had one or
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10 more cytology samples with only normal results, but only in one or two age categories (50-54, 55-59 or 60-
11 293 64); 3) "Long-term unscreened" if no cervical cytology sample at age 50-64; and 4) "Abnormal screening" if
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13 294 women a) had ASC-US or worse at least once at age 50-59 or b) HPV positive at age 60-64.
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17 Screening participation, cervical cytology samples, numbers of colposcopies/conizations performed,
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19 compliance to follow-up among positive self-samplers, and disease outcomes (HPV positivity rate and
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21 histological outcomes) will be estimated as proportions. Participation in the intervention group will be
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23 reported by age groups, screening history, and sampling method (GP versus self-sampling). Regression
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25 analyses will be used to estimate the association between CIN2+ detection in women offered cervical
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27 cancer screening compared to those not offered screening. Both crude and adjusted estimates will be
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29 301 presented with 95% confidence intervals (CIs). The cumulative incidence rates of cervical cancer among
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31 302 women in the intervention and reference groups will be reported, including the distribution of the
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33 303 histological types and FIGO stage of the detected cancers. All statistical analyses will be performed using
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35 304 STATA version 16 (College Station, TX: StataCorp LP).
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38 305 39 40 306 **Sample size**

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42 307 The sample size was determined by the primary outcome (CIN2+). In Denmark, approximately 167,000
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44 308 Danish women are currently aged 65-69 years, 37,000 of whom (22,2%) reside in the CDR (intervention
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46 309 group) (20). We anticipate that an estimated 55% of these will not have a record of a cervical cytology
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48 310 sample in the DPDB within the past five years. As follows, the study cohort will consist of a total of 91,850
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50 311 women, including approximately 20,000 women in the intervention group. We assume that 50% of the
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52 312 eligible women in the intervention group will accept the screening offer and that the proportion of CIN2+ is
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54 313 0.3% among participants. Thus, by including 10,000 women in the intervention group, the study will have a
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56 314 power of 80% to detect a difference in the CIN2+ proportion of 0.1 percentage points between the
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315 intervention and reference group. The proportion of CIN2+ that was chosen (0.3%) is a conservative
316 estimate inspired by Swedish data reporting a CIN2+ proportion of 0.38% among 56-60-year-old women
317 attending HPV-based screening using the Cobas 4800 test (48).

Timeline

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

PATIENT AND PUBLIC INVOLVEMENT

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

ETHICS AND DISSEMINATION

Ethics

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

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337 **Dissemination**

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

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344 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

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

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

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

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371 **Trial status**

372 Ongoing.

373 **Acknowledgements:**

374 Not applicable.

375 **Contributors:**

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

382 **Funding:**

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

385 **Competing interests:**

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

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4 394 **Patient consent:**

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7 395 Not required

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9 396 **Ethics approval:**

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11 397 The study protocol has been submitted to the Ethical Committee which deemed that the study was not
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13 398 notifiable to the Committee and informed consent is therefore not required.

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17 399 **Provenance and peer review:**

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19 400 Not commissioned; externally peer-reviewed.

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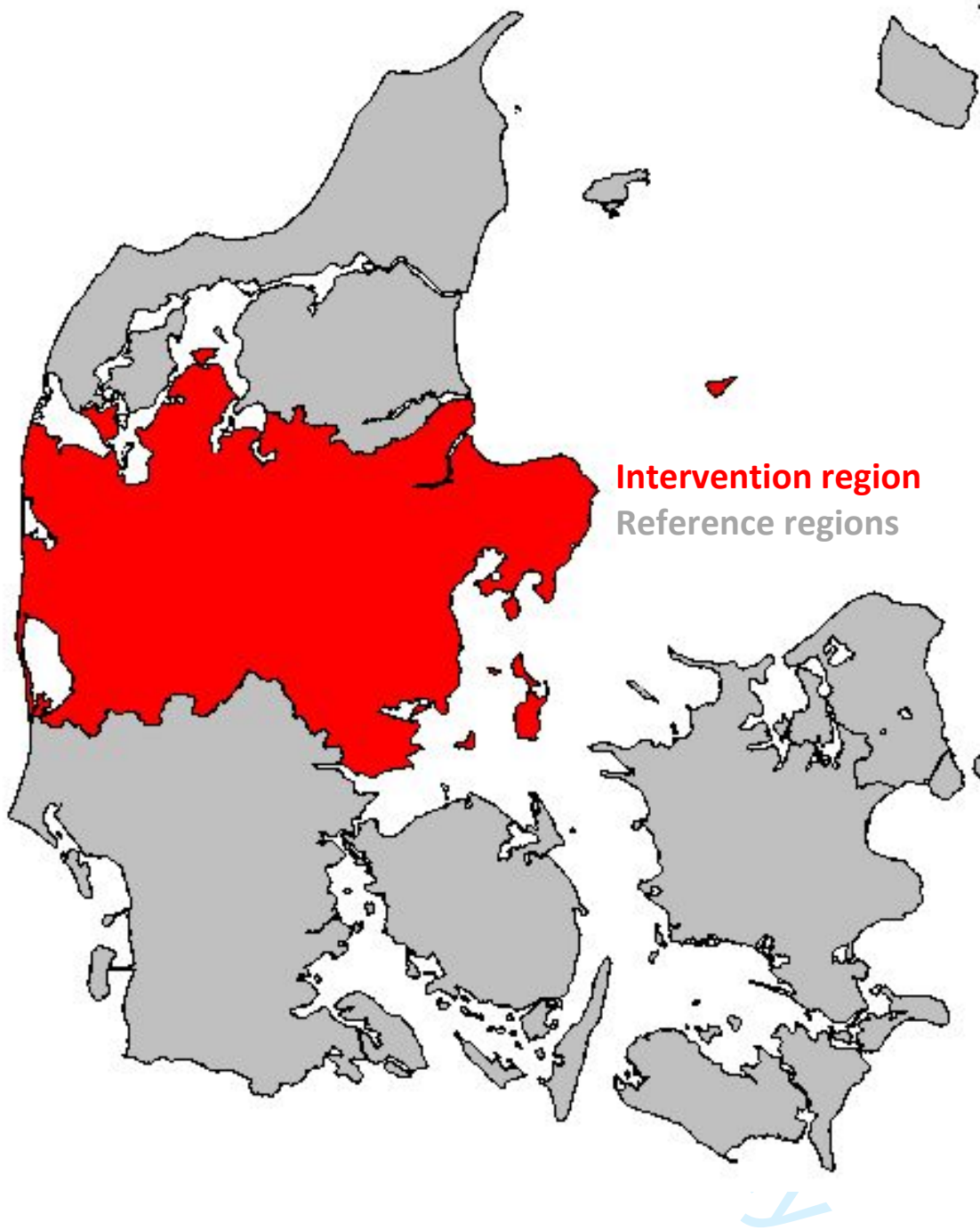
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522 **Table 1: Overview over data sources and information**

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)

523 Table notes: GP: General Practitioner.

Figure 1: Map of the intervention and reference regions



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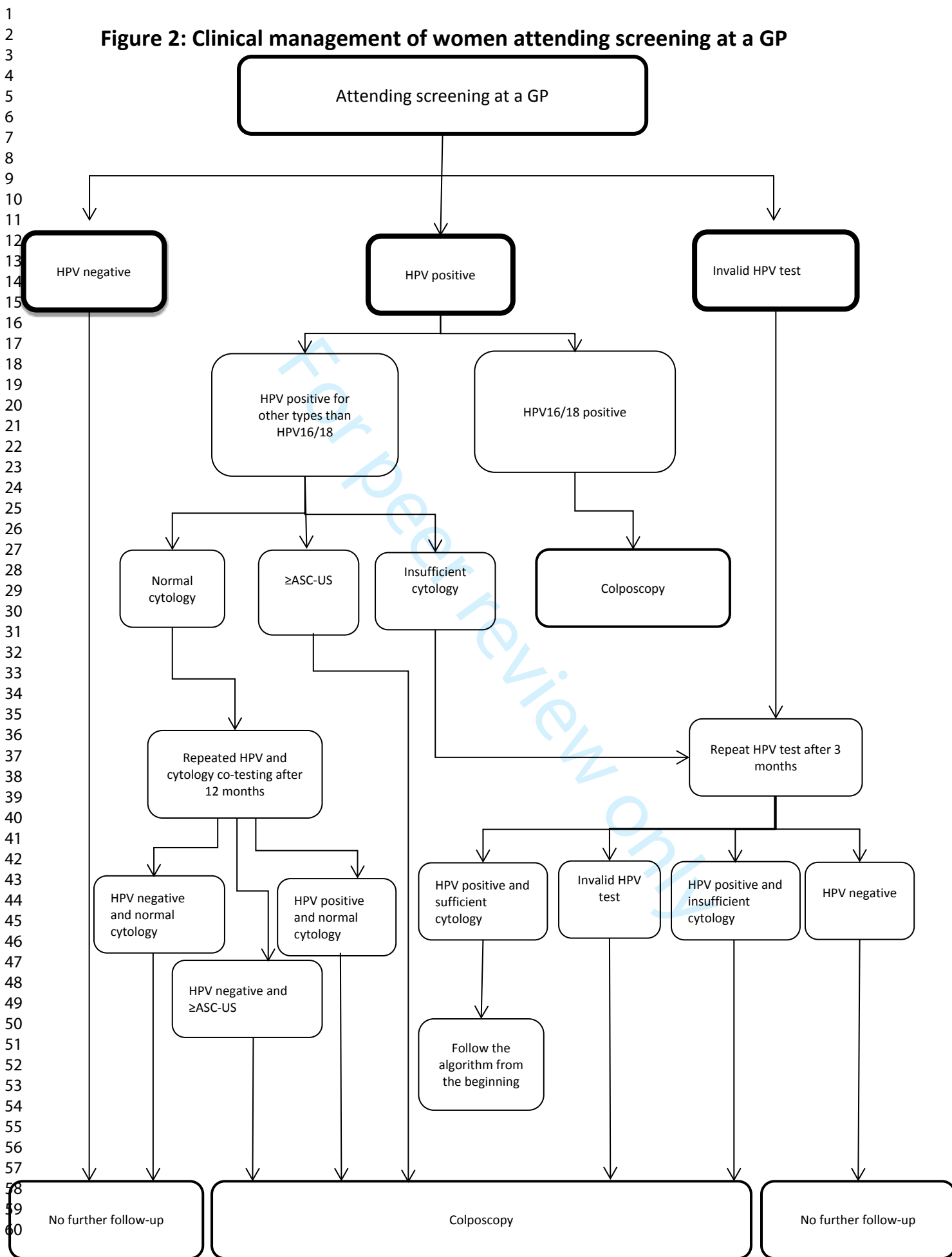
Figure 2: Clinical management of women attending screening at a GP

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

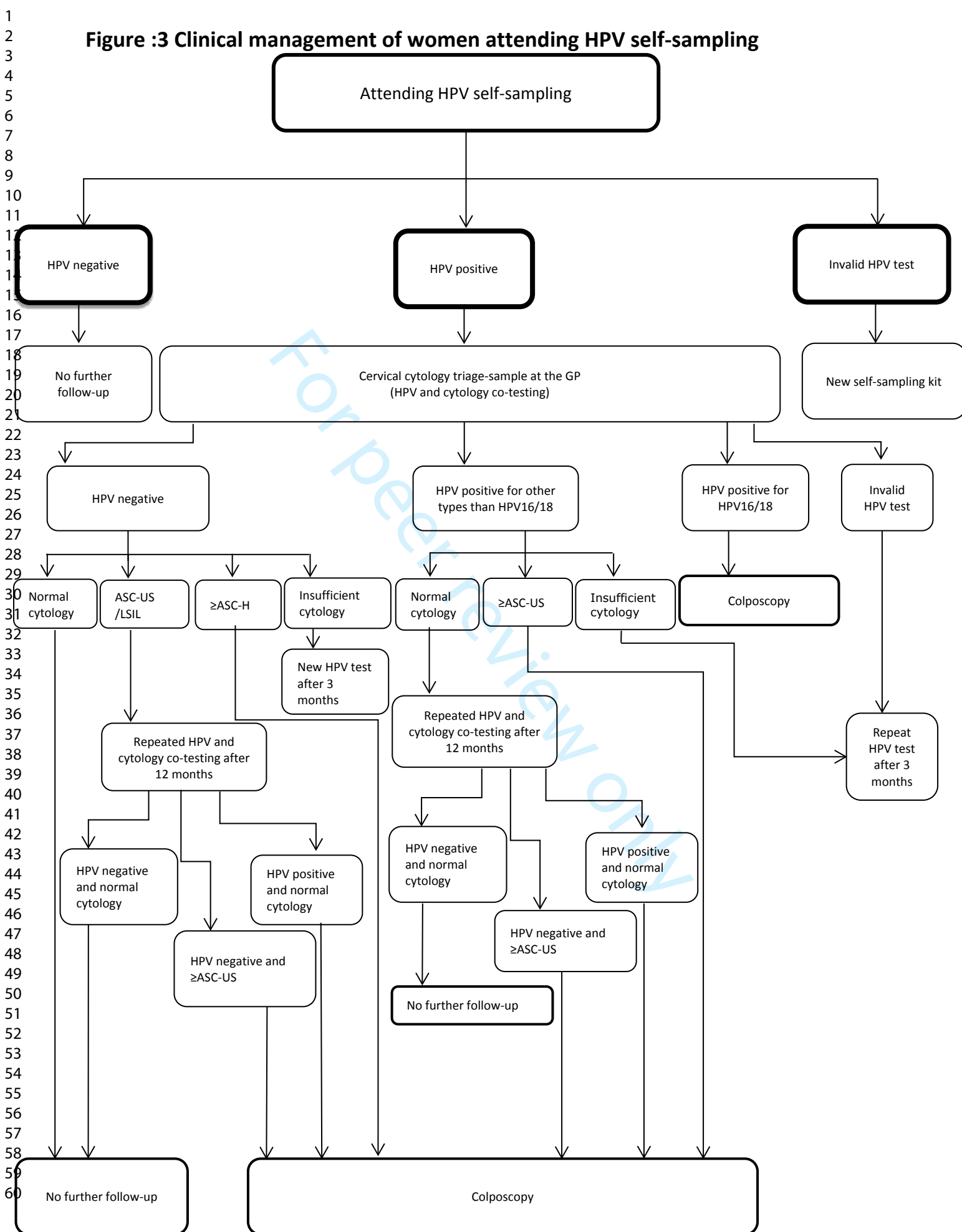


Figure notes: GP: General practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US include: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells cannot 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

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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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48 **ABSTRACT**

50 **Introduction**

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

57 **Methods and Analysis**

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

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

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

70 **Ethics and dissemination**

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

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

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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

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87 INTRODUCTION

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89 Cervical cancer screening ceases between the ages of 60 and 65 in most Western countries¹⁻³. There is no
90 solid evidence about which age and with which criteria to cease screening^{1,4-6}, but the cessation of
91 screening in older women is often justified by a low prevalence of high-risk human papillomavirus (HPV) in
92 women ≥ 55 years^{7,8} and by a concern that the harms of continuing screening may outweigh potential
93 benefits². Many countries with long-established screening programs, including Denmark, experience a
94 second incidence peak of cervical cancer around the age of 75-80 years^{9,10}, with a hysterectomy-corrected
95 incidence rate of 29.4 per 100,000 person-years in women aged 75-79¹¹. These older women are more
96 often diagnosed with advanced-stage disease and mortality due to cervical cancer is high as compared to
97 younger women^{12,13}. It has been hypothesized that the incidence peak could be a result of a mid-life
98 change of sexual partners or reactivation of a latent HPV infection as the immune system weakens with age
99¹⁴⁻¹⁷. However, a recent Danish study of HPV DNA prevalence in women aged 69 and above showed no
100 increase in prevalence that could explain the cervical cancer peak in this age group¹⁸. It has also been
101 hypothesized that the current peak in older ages could be attributed to an insufficient screening history in
102 older birth cohorts¹⁹. Whatever the reason, the increasing female life expectancy (at age 65 years it is
103 about 20 additional years) has raised the question if the upper age limit for screening should be extended
104 to 69 or 70 years^{10,20,21}. Case-control studies have reported benefits of cervical cytology screening at older
105 ages with respect to reduced incidence and mortality^{7,22-25}, even among previously screened women⁴.
106 However, a prospective evaluation of HPV-screening at ages 65-69 in a population-based intervention study
107 including a reference group is missing.
108 The effectiveness of cervical cancer screening among older women will depend on the participation rate
109 and, in particular, the ability to reach long-term unscreened women, as these women have a pronounced
110 risk of cancer^{6,26}. Currently, participation in routine screening decreases with increasing age leaving a
111 relatively high proportion of older women under-screened¹⁰. A potential solution to this challenge could be
112 to offer older women a self-sampling kit for HPV testing (self-sampling). Self-sampling is an accurate and

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4 113 well-accepted screening method, proven superior to physician-based screening in reaching long-term
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6 114 unscreened women ^{27 28}. Yet, it remains unknown whether an older screening population will benefit from a
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9 115 self-sampling offer.
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11 116 **OBJECTIVES**

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14 117 This study will evaluate if expanding the upper screening age to include women aged 65-69-year and
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16 118 inviting them to choose between general practitioner (GP)-based screening or self-sampling results in
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18 119 increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to existing
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20 120 practice where women in this age group of are not invited to routine screening . Furthermore, it will be
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23 121 assessed whether self-sampling is better than GP-based screening in reaching long-term unscreened
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25 122 women.
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27 123 **HYPOTHESES**

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32 125 We hypothesize that expanding the upper screening age will result in increased detection of CIN2+ cases
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34 126 and, long-term, potentially reduce the cervical cancer incidence compared to women not invited to
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37 127 screening. Finally, we hypothesize that self-sampling will be superior to GP-based screening in reaching
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39 128 long-term unscreened women.
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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 region³⁰. 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 years³⁰. 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 history³¹. 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 charge³⁰.

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.

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152 **The call-recall invitation system**

153 The Danish screening program is based on an integrated call-recall system using data from the invitation
154 module in the nationwide Danish Pathology Databank (DPDB)^{30 32 33}. The Conseillers en Gestion et
155 Informatique (CGI) Institute operates the call-recall system and it is designed so that each region only
156 invites women residing in their catchment area. The call-recall system invites women for screening after
157 the age-specific interval has passed since their latest invitation or cervical cytology sample (whichever came
158 last). Samples obtained opportunistically, symptomatically or as part of surveillance are also recorded in the
159 DPDB and postpone the next invitation. The system also keeps track of women who are ineligible for
160 screening because they have actively opted out of the program or have had a hysterectomy. The latter
161 registration is rather incomplete and varies between the regions. In detail, the invitation module links
162 cervical cytology data (Systematized Nomenclature of Medicine, SNOMED codes: T8X3* and T8X210) from
163 the DPDB's main pathology module with information about residency and vital status from the Danish Civil
164 Registration System^{34 35}. Linkage is performed using the unique Civil Personal Registration number (CPR),
165 which is assigned to every Danish citizen upon birth and to residents upon immigration³⁵. The CPR number
166 is used by all citizens for any contact to the Danish health care system.

167 **Design and eligibility criteria**

168 This study is a nationwide prospective population-based non-randomized intervention study (i.e. a quasi-
169 experimental design)³⁶. Women will consecutively be deemed eligible if they meet the following criteria at
170 the time of inclusion: aged 65 to 69 years; resident in Denmark for the past 5 years; no record of a cervical
171 cytology sample or invitation in the past 5 years; not registered in the invitation module as having actively
172 opted out of the screening program or having a record of total hysterectomy or cervical amputation in the
173 Danish National Patient Register³⁷. Eligible women residing in the CDR will be allocated to the intervention
174 group, while women residing in the other four Danish regions will be allocated to the reference group
175 (Figure 1). In the intervention group, the invitation module will be set-up to identify women fitting the

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4 176 inclusion criteria, and simultaneously a comparable list of eligible women in the reference group will be
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6 177 compiled by CGI at the Department of Public Health Programmes' request. Inclusion started in April 2019
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9 178 and eligible women will be identified with six months intervals until the desired number of women have
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11 179 been included. The follow-up period for the included women will start on the date of the invitation and end
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13 180 at time of death, emigration, cervical amputation, total hysterectomy, or end of study. Women that move
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15 181 between the intervention region and reference regions in the follow-up period will subsequently be
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18 182 excluded from the analysis.

21 183 **Intervention group**

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23 184 Women living in the CDR and therefore eligible for the intervention group will be invited to HPV-based
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25 185 cervical cancer screening by either scheduling an appointment for having a cervical cytology sample
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28 186 collected at their GP or collecting a cervico-vaginal sample themselves in their own home using a self-
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30 187 sampling kit. Women will receive an invitation and an information sheet by digital mail, while those
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32 188 exempted from digital mail as per routine will receive the information by postal mail ³⁸. The invitation
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34 189 explains how to request the self-sampling kit and states that once the woman attends screening it will
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37 190 implicitly represent her consent to store her sample for future quality improvement of the screening
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39 191 program. A phone number for calling the study investigator to decline this option will be available. Test
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41 192 results, including follow-up recommendations, will be sent to the women by digital or postal mail and the
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43 193 woman's GP will receive an electronic copy of the test result. Around 98% of all residents in Denmark are
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46 194 listed with a GP ³⁹. As per routine, non-participants will receive up to two reminders at 3 and 6 months post
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48 195 invitation ³⁰. All information will be in Danish.

50 196 **The self-sampling kit**

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52 197 The self-sampling kit can be requested by phone or through a study webpage. After receiving the orders in
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55 198 the department, the kit will be mailed to the women within four business days. The kit includes the dry
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57 199 Evalyn[®] brush self-sampling device (Rovers Medical Devices B.V, Oss, Netherlands) ⁴⁰, written and picture-
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200 based user instructions on how to collect and mail the self-sample, and a pre-stamped return envelope
201 addressed to the Department of Pathology, Randers Regional Hospital ⁴¹. The acceptability of both the self-
202 sampling device and user instructions has been carefully evaluated in previous studies, although among
203 younger women (30-59 years) ^{42 43}.

204 **Processing and analysis of samples**

205 In the intervention group, all samples will be prepared, processed and analyzed at the Department of
206 Pathology, Randers Regional Hospital according to the routine laboratory protocols. All HPV testing will be
207 performed using the clinically validated and Federal Drug Agency (FDA)-approved Cobas[®] 4800 DNA test
208 (Roche Diagnostics, Switzerland) ⁴⁴, as this is the routine test platform used in the CDR. The test is an
209 automated real-time PCR-based test designed to detect high-risk HPV types: 16,18,31,33,35,39,45,51,52,
210 56,58,59,66 and 68 ^{44 45} and is validated for use on SurePath collected samples ⁴⁶. Results will be reported as
211 1) HPV negative, 2) HPV positive (HPV16, HPV18 and/ or other HPV types) or 3) invalid ⁴¹. All samples with
212 an invalid test result will be re-tested, and the second result will be considered definitive. The Cobas test
213 measures beta-globin as an internal control for sample cellularity, valid sample extraction, and
214 amplification ⁴⁵.

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

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4 224 Evalyn brush device will be rinsed into 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and
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6 225 processed as previously described ⁴².
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9 226 From the cervical cytology samples and self-samples, 2 mL of the SurePath medium will be placed in test
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11 227 tubes for HPV testing. The residual eluate material from these samples will be stored at -80°C for future
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13 228 extended genotyping and DNA methylation analysis. As part of the study, and only in the intervention
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15 229 group, p16/Ki67 cytology dual staining (CINtec[®] PLUS cytology kit, Roche Diagnostics, Switzerland) will be
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17 230 performed consecutively on the residual SurePath cell-pellet obtained from women with an HPV positive
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20 231 cervical cytology sample and sufficient material for cytology testing. The dual staining result will not affect
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22 232 the clinical management of the woman. Except for the dual staining result, all test results will routinely be
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24 233 registered in the DPDB ³³.
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26 234 **Clinical management**

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29 235 Figures 2 and 3 show the recommended, and therefore, expected clinical management for women in the
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31 236 intervention group, but management may deviate depending on the clinical presentation of the individual
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33 237 woman. The recommendations are in accordance with the routine screening guidelines for 60-64-year-old
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35 238 women and the new guidelines for clinical management of older women with dysplasia and HPV ^{31 47}.
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38 239 Women who are positive for HPV16 or 18 AND other types will be managed similar to HPV16/18 positive
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40 240 women.
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42 241 For women attending GP-based screening, those who tested HPV negative will have no further follow-up
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44 242 (Figure 2). Women tested positive for HPV 16/18 will be referred directly to colposcopy (regardless of the
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46 243 cytology result). Women tested HPV positive for other types than HPV16/18 with ASC-US or more severe
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48 244 cytological abnormalities will be referred to colposcopy, while women with HPV types other than HPV16/18
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50 245 and normal cytology will undergo repeated co-testing (HPV and cytology) after 12 months and will be
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52 246 referred for colposcopy if either test result is positive.
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56 247 Figure 3 presents the follow-up recommendations for women attending self-sampling. Women who tested
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58 248 HPV negative in their self-sample will have no further follow-up. Women with an HPV positive self-sample
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(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 guidelines⁴⁸. Some women may also undergo a diagnostic conization as part of a clinical "See and Treat" study⁴⁹. 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 assay¹⁸ 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}.

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274 **Outcomes**

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

276 In both groups, the primary outcome will be the proportion of CIN2+ (CIN2, CIN3/AIS, and cancer) detected
277 within 18 months following registration of a cervical cytology sample or self-sample. The proportion of
278 CIN3+ (CIN3/AIS, and cancer) will be a secondary outcome. The most severe histological test result will be
279 used if more than one result is available in the follow-up period. Other outcomes in the intervention group
280 will be screening participation measured 12 months after the invitation date, defined by returning a self-
281 sample or having a cervical cytology taken; screening history of participants, stratified by sampling
282 procedure; HPV positivity rate and HPV type distribution in self-samples versus GP-collected cervical
283 cytology samples; cytology results, and the percentage of HPV positive self-samplers undergoing
284 appropriate follow-up. Compliance to follow-up after self-sampling will be defined as attending a GP for a
285 cervical cytology-triage sample within 30, 60, 90 or 180 days after mailing of the test results. The
286 proportion and results of cervical cytology samples obtained among women not invited for screening will
287 be identified in the reference group and measured 12 months post invitation date. As in another study²,
288 the primary measure of harms will in both groups be the number of colposcopies/conizations performed,
289 both overall and relatively to \leq CIN1, CIN2, CIN3/AIS, and cancers detected within a follow-up period of 18
290 months after registration of a cervical cytology sample or self-sample. Long-term outcomes will be cervical
291 cancer incidence rates reported by groups at 5- and 10-years post invitation dates. A description of
292 histological type and FIGO stage of the detected cervical cancers will be provided.

293 **Data sources and statistical analysis**

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

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

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299 and the HPV-exit test at age 60-64. The categorization of screening history is expected to be as follows ⁶:

300 1) "Sufficiently screened with normal results" if women had a) at least one normal cytology at age 50-54

301 and b) at least one normal cytology at age 55-59, and c) no abnormal cytology (ASC-US or worse) at age 50-

302 59, and d) HPV negative at age 60-64; 2) "Insufficiently screened with normal results" if women had one or

303 more cytology samples with only normal results, but only in one or two age categories (50-54, 55-59 or 60-

304 64); 3) "Long-term unscreened" if no cervical cytology sample at age 50-64; and 4) "Abnormal screening" if

305 women a) had ASC-US or worse at least once at age 50-59 or b) HPV positive at age 60-64.

306 Screening participation, cervical cytology samples, numbers of colposcopies/conizations performed,

307 compliance to follow-up among positive self-samplers, and disease outcomes (HPV positivity rate and

308 histological outcomes) will be estimated as proportions. Participation in the intervention group will be

309 reported by age groups, screening history, and sampling method (GP versus self-sampling). Regression

310 analyses will be used to estimate the association between CIN2+ detection in women offered cervical

311 cancer screening compared to those not offered screening. Both crude and adjusted estimates will be

312 presented with 95% confidence intervals (CIs).The cumulative incidence rates of cervical cancer among

313 women in the intervention and reference groups will be reported, including the distribution of the

314 histological types and FIGO stage of the detected cancers. All statistical analyses will be performed using

315 STATA version 16 (College Station, TX: StataCorp LP).

316 **Sample size**

317 The sample size was determined by the primary outcome (CIN2+). In Denmark, approximately 167,000

318 Danish women are currently aged 65-69 years, 37,000 of whom (22,2%) reside in the CDR (intervention

319 group) ²¹. We anticipate that an estimated 55% of these will not have a record of a cervical cytology sample

320 in the DPDB within the past five years. As follows, the study cohort will consist of a total of 91,850 women,

321 including approximately 20,000 women in the intervention group. We assume that 50% of the eligible

322 women in the intervention group will accept the screening offer and that the proportion of CIN2+ is 0.3%

323 among participants. Thus, by including 10,000 women in the intervention group, the study will have a

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4 324 power of 80% to detect a difference in the CIN2+ proportion of 0.1 percentage points between the
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6 325 intervention and reference group. The proportion of CIN2+ that was chosen (0.3%) is a conservative
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9 326 estimate inspired by Swedish data reporting a CIN2+ proportion of 0.38% among 56-60-year-old women
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11 327 attending HPV-based screening using the Cobas 4800 test ⁵⁰.

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14 328 **Timeline**

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16 329 The study enrollment is expected to continue until 10,000 participants have been included in the
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19 330 intervention group. Invitations will be sent out prospectively over an expected 4.5 year-period starting
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21 331 April 2019.

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24 332 **PATIENT AND PUBLIC INVOLVEMENT**

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27 333 The research questions were developed in response to the on-going public and scientific discussion in
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29 334 Denmark regarding expanding the upper screening age in the organized cervical cancer screening program.
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31 335 No patients or patient organizations were involved in the development, design, or implementation of this
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34 336 study.

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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.

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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-test⁵¹. 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 Denmark³³, 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. Age⁶, screening history⁶, comorbidities⁵², education level⁶, marital status⁶, smoking status⁷, and sexual behavior⁶ 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

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380 case, it may affect the impact of the intervention on CIN2+ detection rates. Fortunately, we will be able to
381 take into account these potential regional differences by using data from the nationwide DPDB.
382 Detection of invasive and advanced cervical cancer is the optimal outcome measure to evaluate the effect
383 of screening at older ages ⁵¹, but given the relative rarity of cervical cancer in older women, the length of
384 follow-up needed and the large sample size required, we chose CIN2+ and CIN3+ as the primary and
385 secondary outcome, respectively. Yet, it should be noted that the majority of CIN2 and CIN3 lesions
386 detected after age 65 might not have sufficient time to progress to invasive cancer in the remaining
387 lifespan ². For screening purposes, including CIN2+ and CIN3 cases as the primary and secondary outcomes,
388 respectively, may be justified by them being treatable endpoints (conization) in older non-reproductive
389 women according to Danish guidelines ⁴⁷, while still recognizing that the detection and treatment of CIN3,
390 especially CIN2, may be considered as overtreatment.

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392 **Trial status**

393 Ongoing.

394 **Acknowledgements:**

395 Not applicable.

396 **Contributors:**

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

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405 Regional Hospital, which is located in the Central Denmark Region. Some public funding had been provided
406 by the Health Foundation (grant no.:18-B-0125) and other fundraising is on-going. The Health foundation
407 had no role in the design of the study and collection, analysis, and interpretation of the data, and in writing
408 the manuscript.

410 **Competing interests:**

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

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

Patient consent:

420 Not required

Ethics approval:

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

Provenance and peer review:

425 Not commissioned; externally peer-reviewed.

For peer review only

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4 578 **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

579 Table notes: GP: General Practitioner. CIN2+: CIN2, CIN3/AIS, and cancer. CIN3+: CIN3/AIS, and cancer.
580 *)Only available for women with a HPV positive GP-sample or GP-triage sample following a HPV positive self-sample.
581

582 **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 reference 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)

583 Table notes: GP: General Practitioner.

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586 **Figure legends:**

587 Figure 1: Map of the intervention and reference regions

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

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

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Figure 1:

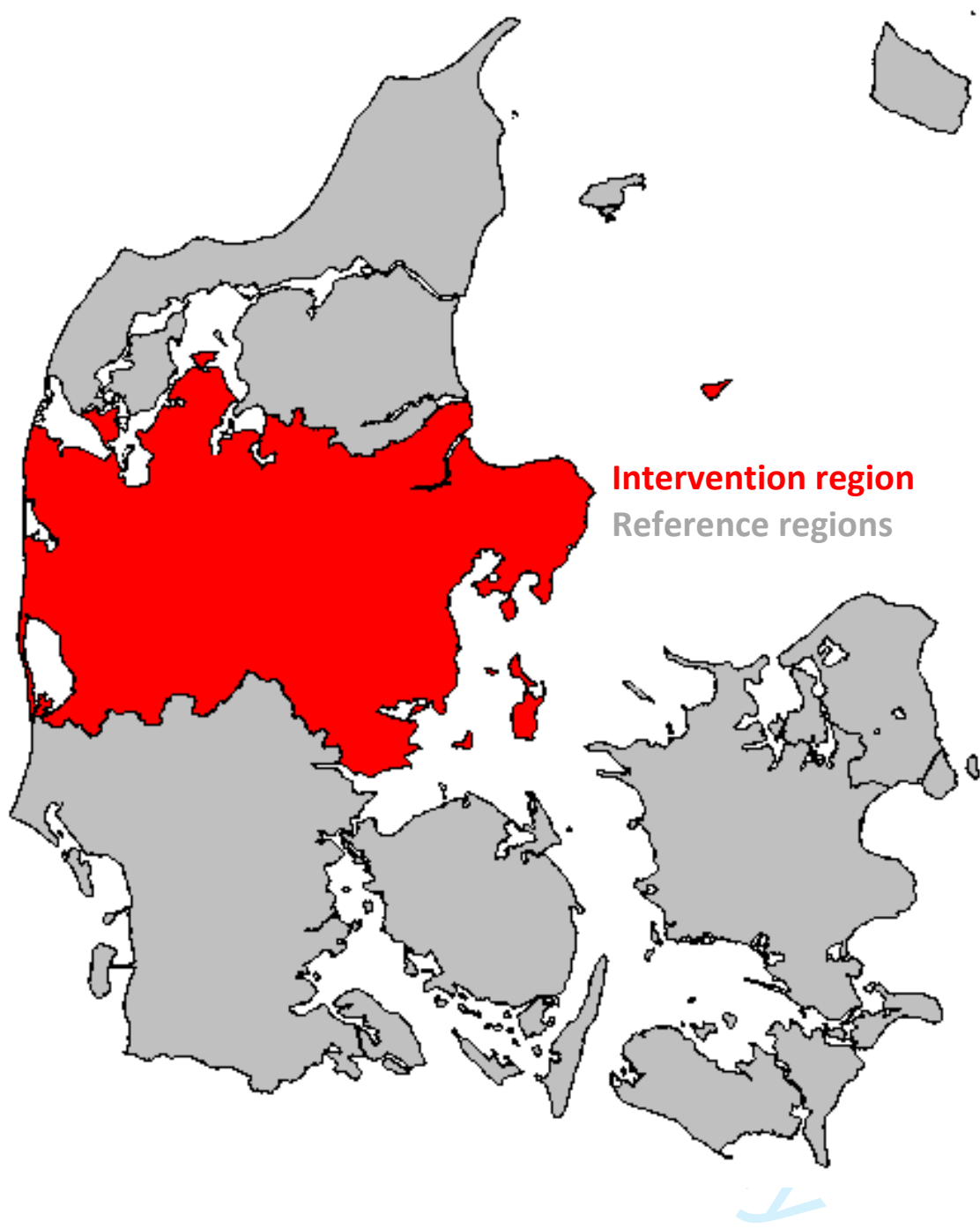


Figure 2:

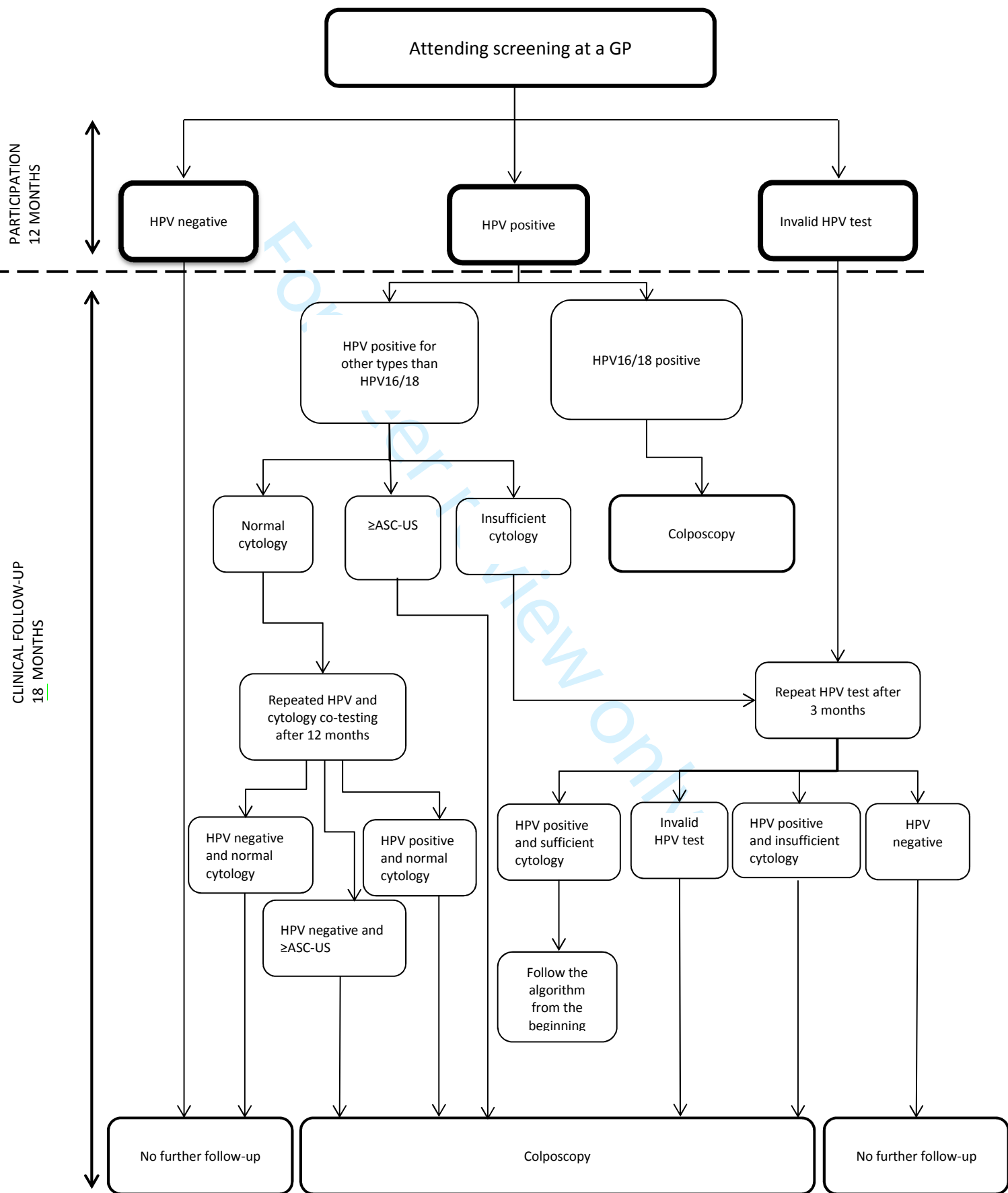


Figure notes: GP: General Practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. ≥ASC-US includes: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells 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:

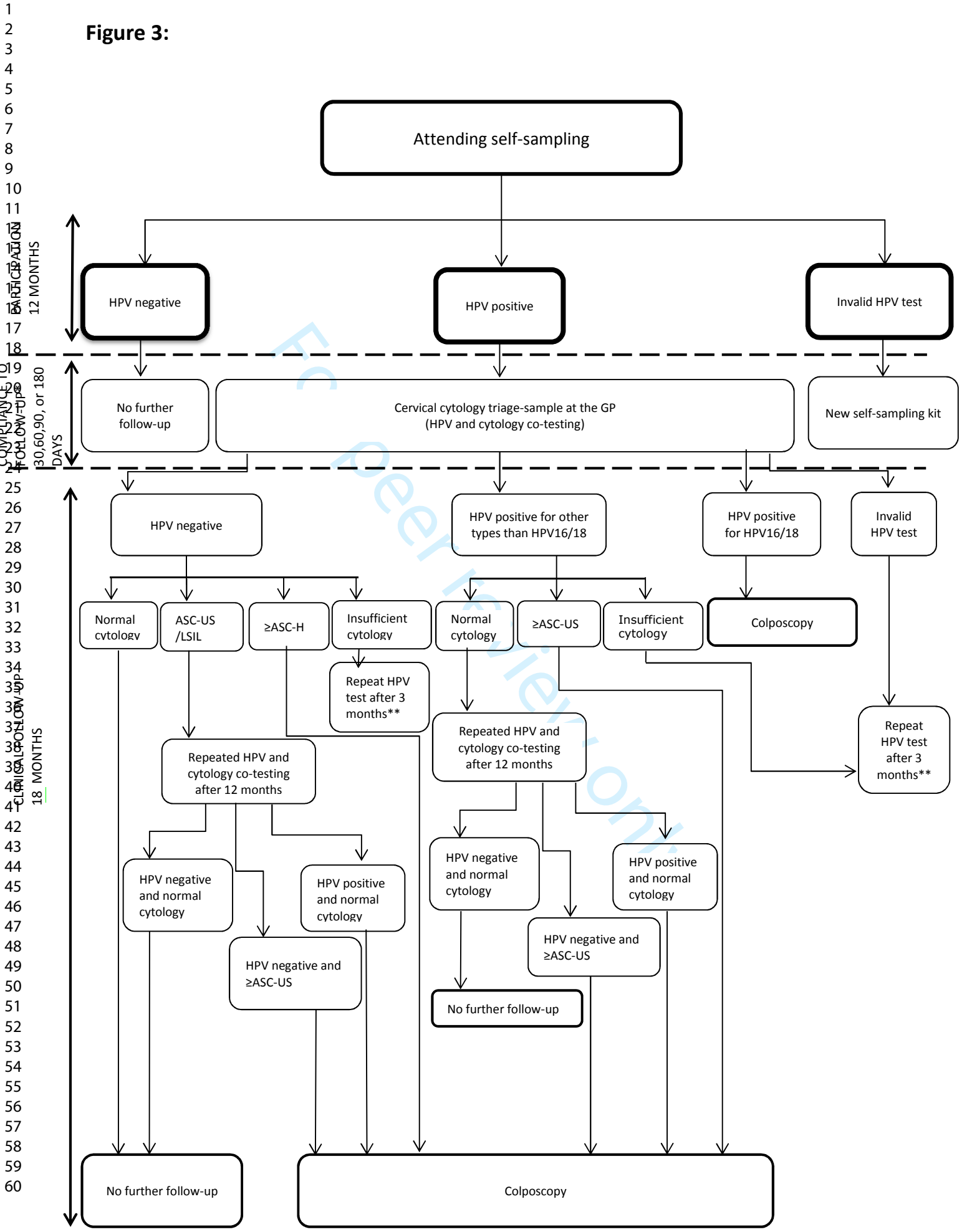


Figure notes: GP: General practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US include: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells of Undetermined Significance (ASC-US); 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. *) Compliance to follow-up among HPV positive self-samplers. **) Follows the same algorithm as shown in Figure 2 among women having repeating HPV testing after 3 months.

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

STUDY

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

48 **ABSTRACT**

50 **Introduction**

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

57 **Methods and Analysis**

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

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

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

70 **Ethics and dissemination**

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

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

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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

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87 INTRODUCTION

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89 Cervical cancer screening ceases between the ages of 60 and 65 in most Western countries¹⁻³. There is no
90 solid evidence about which age and with which criteria to cease screening^{1,4-6}, but the cessation of
91 screening in older women is often justified by a low prevalence of high-risk human papillomavirus (HPV) in
92 women ≥ 55 years^{7,8} and by a concern that the harms of continuing screening may outweigh potential
93 benefits². Many countries with long-established screening programs, including Denmark, experience a
94 second incidence peak of cervical cancer around the age of 75-80 years^{9,10}, with a hysterectomy-corrected
95 incidence rate of 29.4 per 100,000 person-years in women aged 75-79¹¹. These older women are more
96 often diagnosed with advanced-stage disease, and mortality due to cervical cancer is high as compared to
97 younger women^{12,13}. It has been hypothesized that the incidence peak at older ages could be a result of a
98 mid-life change of sexual partners or reactivation of a latent HPV infection as the immune system weakens
99 with age¹⁴⁻¹⁷. However, a recent Danish study of HPV DNA prevalence in women aged 69 and above
100 showed no increase in prevalence that could explain the cervical cancer peak at older ages¹⁸. The authors
101 stated that this result may be explained by the fact that an HPV infection may become undetectable at a
102 late stage in the oncogenic process^{18,19}. It has also been hypothesized that the current peak in older ages
103 could be attributed to an insufficient screening history in older birth cohorts²⁰. Whatever the reason, the
104 increasing female life expectancy (at age 65 years it is about 20 additional years) has raised the question if
105 the upper age limit for screening should be extended to 69 or 70 years^{10,21,22}. Case-control studies have
106 reported benefits of cervical cytology screening at older ages with respect to reduced incidence and
107 mortality^{7,23-26}, even among previously screened women⁴. However, a prospective evaluation of HPV-
108 screening at ages 65-69 in a population-based intervention study including a reference group is missing.
109 The effectiveness of cervical cancer screening among older women will depend on the participation rate
110 and, in particular, the ability to reach long-term unscreened women, as these women have a pronounced
111 risk of cancer^{6,27}. Currently, participation in routine screening decreases with increasing age leaving a
112 relatively high proportion of older women under-screened¹⁰. A potential solution to this challenge could be

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4 113 to offer older women a self-sampling kit for HPV testing (self-sampling). Self-sampling is an accurate and
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6 114 well-accepted screening method, proven superior to physician-based screening in reaching long-term
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9 115 unscreened women ^{28 29}. Yet, it remains unknown whether an older screening population will benefit from a
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11 116 self-sampling offer.

13 117 **OBJECTIVES**

16 118 This study will evaluate if expanding the upper screening age to include women aged 65-69-year and
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18 119 inviting them to choose between general practitioner (GP)-based screening or self-sampling results in
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20 120 increased detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) compared to existing
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23 121 practice where women in this age group are not invited to routine screening. Furthermore, it will be
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25 122 assessed whether self-sampling is better than GP-based screening in reaching long-term unscreened
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27 123 women.

32 125 **HYPOTHESES**

34 126 We hypothesize that expanding the upper screening age will result in increased detection of CIN2+ cases
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37 127 and, long-term, potentially reduce the cervical cancer incidence compared to women not invited to
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39 128 screening. Finally, we hypothesize that self-sampling will be superior to GP-based screening in reaching
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41 129 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 region³¹. 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 years³¹. 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 history³². 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 charge³¹.

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 immigration³⁶. 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 Register³⁸. 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

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4 177 inclusion criteria, and simultaneously a comparable list of eligible women in the reference group will be
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6 178 compiled by CGI at the Department of Public Health Programmes' request. Inclusion started in April 2019
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9 179 and eligible women will be identified with six months' intervals until the desired number of women have
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11 180 been included. The follow-up period for the included women will start on the date of the invitation and end
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13 181 at time of death, emigration, cervical amputation, total hysterectomy, or end of study. Women that move
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15 182 between the intervention region and reference regions in the follow-up period will subsequently be
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18 183 excluded from the analysis.
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21 184 **Intervention group**

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23 185 Women living in the CDR and therefore eligible for the intervention group will be invited to HPV-based
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25 186 cervical cancer screening by either scheduling an appointment for having a cervical cytology sample
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28 187 collected at their GP or collecting a cervico-vaginal sample themselves in their own home using a self-
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30 188 sampling kit. Women will receive an invitation and an information sheet by digital mail, while those
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32 189 exempted from digital mail as per routine will receive the information by postal mail ³⁹. The invitation
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34 190 explains how to request the self-sampling kit and states that once the woman attends screening it will
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37 191 implicitly represent her consent to store her sample for future quality improvement of the screening
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39 192 program. A phone number for calling the study investigator to decline this option will be available. Test
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41 193 results, including follow-up recommendations, will be sent to the women by digital or postal mail and the
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43 194 woman's GP will receive an electronic copy of the test result. Around 98% of all residents in Denmark are
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45 195 listed with a GP ⁴⁰. As per routine, non-participants will receive up to two reminders at 3 and 6 months post
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48 196 invitation ³¹. All information will be in Danish.
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50 197 **The self-sampling kit**

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52 198 The self-sampling kit can be requested by phone or through a study webpage. After receiving the orders in
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55 199 the department, the kit will be mailed to the women within four business days. The kit includes the dry
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57 200 Evalyn[®] brush self-sampling device (Rovers Medical Devices B.V, Oss, Netherlands) ⁴¹, written and picture-
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201 based user instructions on how to collect and mail the self-sample, and a pre-stamped return envelope
202 addressed to the Department of Pathology, Randers Regional Hospital ⁴². The acceptability of both the self-
203 sampling device and user instructions has been carefully evaluated in previous studies, although among
204 younger women (30-59 years) ^{43 44}.

205 **Processing and analysis of samples**

206 In the intervention group, all samples will be prepared, processed and analyzed at the Department of
207 Pathology, Randers Regional Hospital according to the routine laboratory protocols. All HPV testing will be
208 performed using the clinically validated and Federal Drug Agency (FDA)-approved Cobas[®] 4800 DNA test
209 (Roche Diagnostics, Switzerland) ⁴⁵, as this is the routine test platform used in the CDR. The test is an
210 automated real-time PCR-based test designed to detect high-risk HPV types: 16,18,31,33,35,39,45,51,52,
211 56,58,59,66 and 68 ^{45 46} and is validated for use on SurePath collected samples ⁴⁷. Results will be reported as
212 1) HPV negative, 2) HPV positive (HPV16, HPV18 and/ or other HPV types) or 3) invalid ⁴². All samples with
213 an invalid test result will be re-tested, and the second result will be considered definitive. The Cobas test
214 measures beta-globin as an internal control for sample cellularity, valid sample extraction, and
215 amplification ⁴⁶.

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

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4 225 Evalyn brush device will be rinsed into 10 mL SurePath medium (BD Diagnostics, Burlington, NC) and
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6 226 processed as previously described ⁴³.
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9 227 From the cervical cytology samples and self-samples, 2 mL of the SurePath medium will be placed in test
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11 228 tubes for HPV testing. The residual eluate material from these samples will be stored at -80°C for future
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13 229 extended genotyping and DNA methylation analysis. As part of the study, and only in the intervention
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15 230 group, p16/Ki67 cytology dual staining (CINtec[®] PLUS cytology kit, Roche Diagnostics, Switzerland) will be
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18 231 performed consecutively on the residual SurePath cell-pellet obtained from women with an HPV positive
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20 232 cervical cytology sample and sufficient material for cytology testing. The dual staining result will not affect
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22 233 the clinical management of the woman. Except for the dual staining result, all test results will routinely be
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24 234 registered in the DPDB ³⁴.

26 235 **Clinical management**

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29 236 Figures 2 and 3 show the recommended, and therefore, expected clinical management for women in the
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31 237 intervention group, but management may deviate depending on the clinical presentation of the individual
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34 238 woman. The recommendations are in accordance with the routine screening guidelines for 60-64-year-old
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36 239 women and the new guidelines for clinical management of older women with dysplasia and HPV ^{32 48}.
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38 240 Women who are positive for HPV16 or 18 AND other types will be managed similar to HPV16/18 positive
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40 241 women.
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42 242 For women attending GP-based screening, those who tested HPV negative will have no further follow-up
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45 243 (Figure 2). Women tested positive for HPV 16/18 will be referred directly to colposcopy (regardless of the
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47 244 cytology result). Women tested HPV positive for other types than HPV16/18 with ASC-US or more severe
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49 245 cytological abnormalities will be referred to colposcopy, while women with HPV types other than HPV16/18
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51 246 and normal cytology will undergo repeated co-testing (HPV and cytology) after 12 months and will be
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53 247 referred for colposcopy if either test result is positive.
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56 248 Figure 3 presents the follow-up recommendations for women attending self-sampling. Women who tested
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58 249 HPV negative in their self-sample will have no further follow-up. Women with an HPV positive self-sample
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(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 guidelines⁴⁹. Some women may also undergo a diagnostic conization as part of a clinical "see-and-treat" study⁵⁰. 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 assay¹⁸ 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}.

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275 **Outcomes**

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

277 In both groups, the primary outcome will be the proportion of CIN2+ (CIN2, CIN3/AIS, and cancer) detected
278 within 18 months following registration of a cervical cytology sample or self-sample. The proportion of
279 CIN3+ (CIN3/AIS, and cancer) will be a secondary outcome. The most severe histological test result will be
280 used if more than one result is available in the follow-up period. Other outcomes in the intervention group
281 will be screening participation measured 12 months after the invitation date, defined by returning a self-
282 sample or having a cervical cytology taken; screening history of participants, stratified by sampling
283 procedure; HPV positivity rate and HPV type distribution in self-samples versus GP-collected cervical
284 cytology samples; cytology results, and the percentage of HPV positive self-samplers undergoing
285 appropriate follow-up. Compliance to follow-up after self-sampling will be defined as attending a GP for a
286 cervical cytology-triage sample within 30, 60, 90 or 180 days after mailing of the test results. The
287 proportion and results of cervical cytology samples obtained among women not invited for screening will
288 be identified in the reference group and measured 12 months post invitation date. As in another study²,
289 the primary measure of harms will in both groups be the number of colposcopies/conizations performed,
290 both overall and relatively to \leq CIN1, CIN2, CIN3/AIS, and cancers detected within a follow-up period of 18
291 months after registration of a cervical cytology sample or self-sample. Long-term outcomes will be cervical
292 cancer incidence rates reported by groups at 5- and 10-years post invitation dates. A description of
293 histological type and FIGO stage of the detected cervical cancers will be provided.

294 **Data sources and statistical analysis**

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

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

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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

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

329 **Timeline**

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

333 **PATIENT AND PUBLIC INVOLVEMENT**

334 The research questions were developed in response to the on-going public and scientific discussion in
335 Denmark regarding expanding the upper screening age in the organized cervical cancer screening program.
336 No patients or patient organizations were involved in the development, design, or implementation of this
337 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.

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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-test⁵².

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 Denmark³⁴, 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. Age⁶, screening history⁶, comorbidities⁵³, education level⁶, marital status⁶, smoking status⁷, and sexual behavior⁶ 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

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

384 Detection of invasive and advanced cervical cancer is the optimal outcome measure to evaluate the effect
385 of screening at older ages ⁵², but given the relative rarity of cervical cancer in older women, the length of
386 follow-up needed and the large sample size required, we chose CIN2+ and CIN3+ as the primary and
387 secondary outcome, respectively. Yet, it should be noted that the majority of CIN2 and CIN3 lesions
388 detected after age 65 might not have sufficient time to progress to invasive cancer in the remaining
389 lifespan ². For screening purposes, including CIN2+ and CIN3 cases as the primary and secondary outcomes,
390 respectively, may be justified by them being treatable endpoints (conization) in older non-reproductive
391 women according to Danish guidelines ⁴⁸, while still recognizing that the detection and treatment of CIN3,
392 and especially CIN2, may be considered as overtreatment, because an unknown proportion of these lesions
393 would never have progressed to cancer in the woman's lifetime⁵⁵. Specifically, it is important to take into
394 account that conization is associated with an increased risk of bleeding and stenosis, which may hinder or
395 challenge sampling from the cervix post-conization⁵², and that false-positive screening results may place
396 some women in a surveillance cycle of unclear end, which may cause distress⁵⁵.

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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.

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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

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421 editorial rights pertaining to this manuscript. The authors retained the right to submit the manuscript. MT,
422 JB, JSJ and BA have participated in other studies with HPV test kits sponsored by Roche and self-sampling
423 devices sponsored by Axlab. MT has received honoraria from Roche Diagnostics and AstraZeneca for
424 lectures on HPV self-sampling and HPV triage-methods, respectively. AH has received lecture fees from
425 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.

For peer review only

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610 **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	Intervention group
HPV positive self-samplers	
Proportion and results of cervical	Reference group
cytology samples	
Colposcopies and conizations	Intervention vs. reference group
Cervical cancer incidence	Intervention vs. reference group

611 Table notes: GP: General Practitioner. CIN2+: CIN2, CIN3/AIS, and cancer. CIN3+: CIN3/AIS, and cancer.

612 *)Only available for women with a HPV positive GP-sample or GP-triage sample following a HPV positive self-sample.

614 **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)

615 Table notes: GP: General Practitioner.

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618 **Figure legends:**

619 Figure 1: Map of the intervention and reference regions

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

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

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Figure 1:

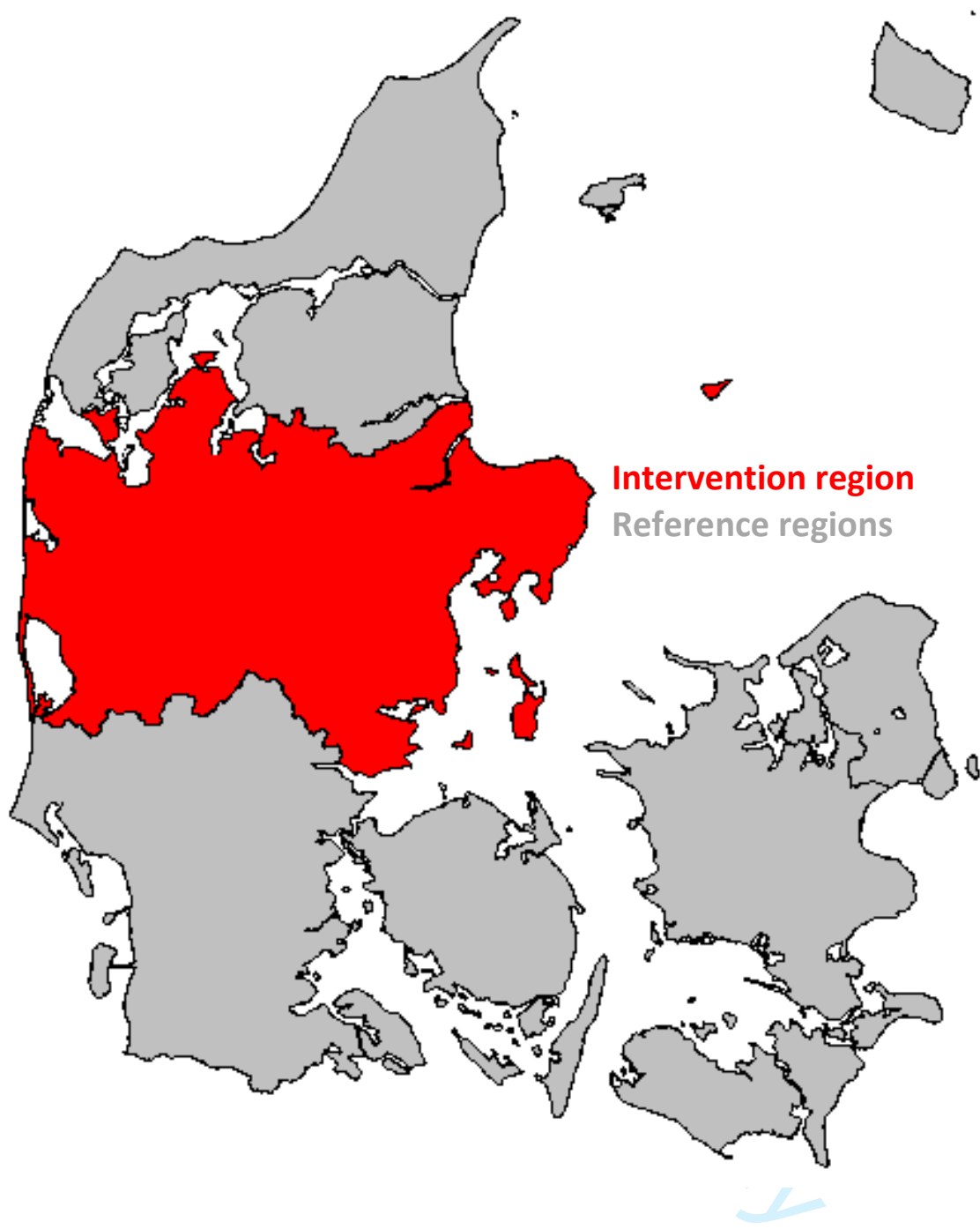


Figure 2:

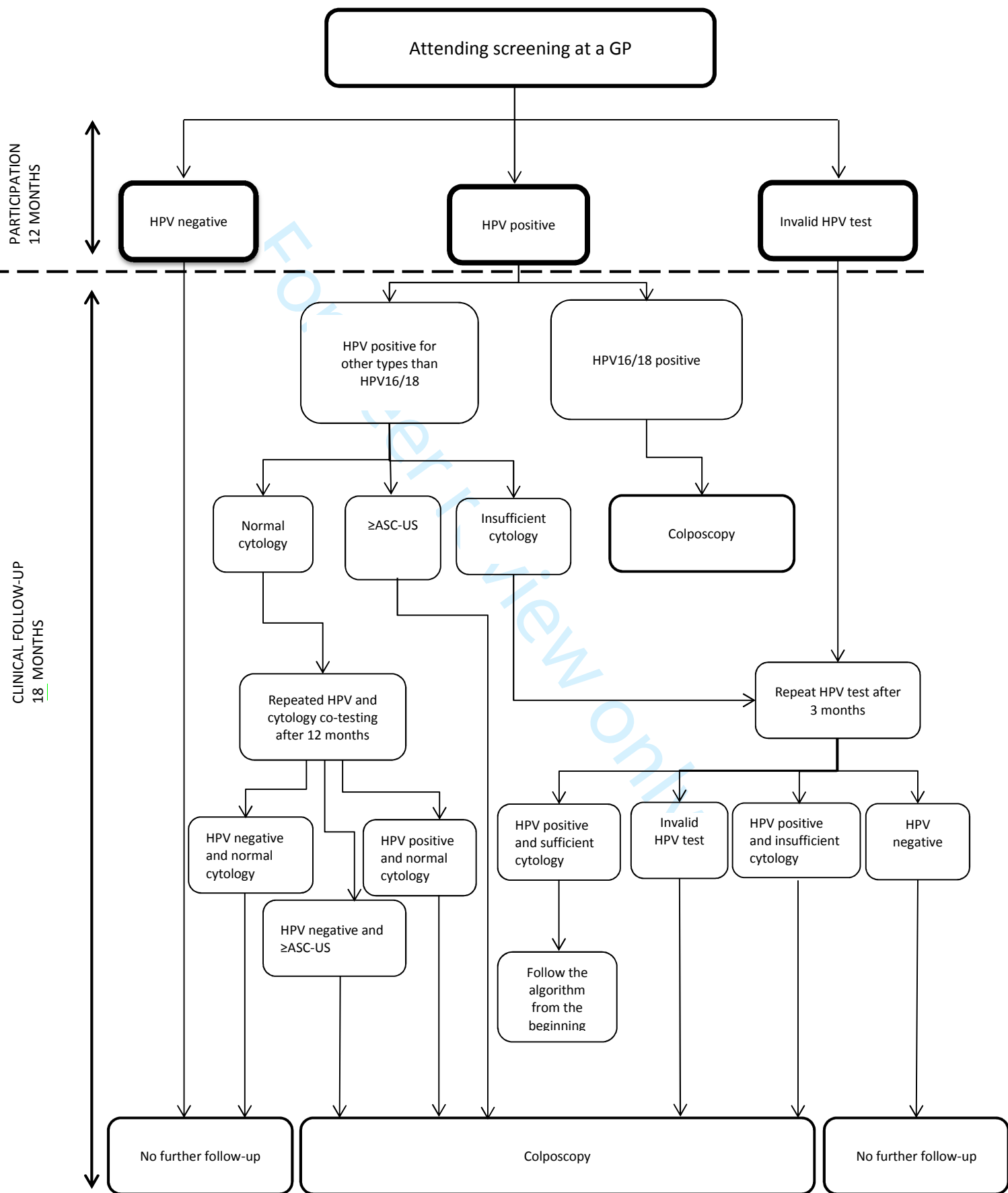


Figure notes: GP: General Practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. ≥ASC-US includes: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells 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:

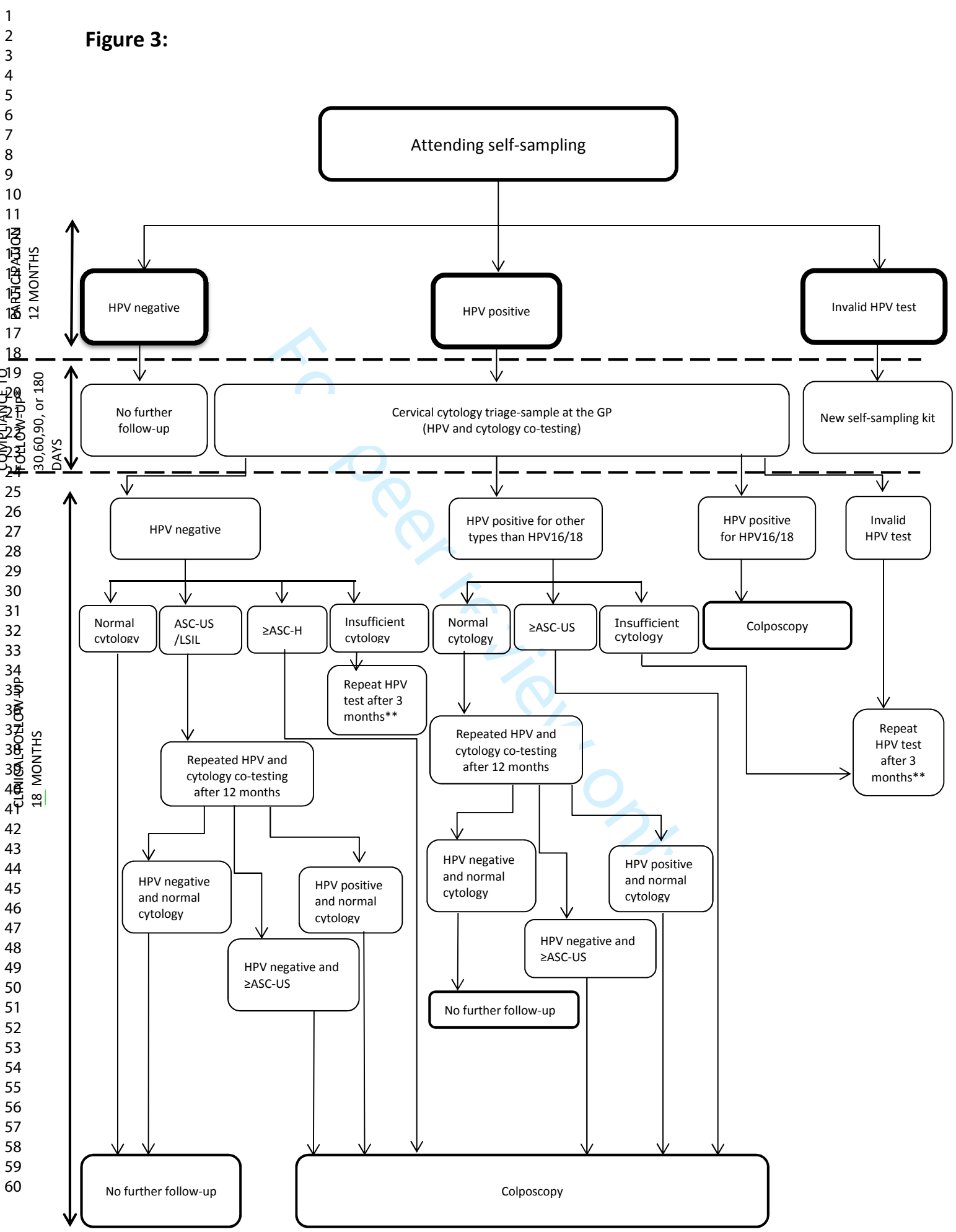


Figure notes: GP: General practitioner. HPV: Human Papillomavirus. HPV other types than HPV16/18: 31,33,35,39,45,51,52, 56,58,59,66 and 68. ≥ASC-US include: Atypical Squamous Cells of Undetermined Significance (ASC-US); Low-grade Squamous Intraepithelial Lesion (LSIL); Atypical Squamous Cells (ASC-H); 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. *) Compliance to follow-up among HPV positive self-samplers. **) Follows the same algorithm as shown in Figure 2 among women having repeating HPV testing after 3 months.