Randomised trial of HPV self-sampling among non-attenders in the Slovenian cervical screening programme ZORA: comparing three different screening approaches

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Supplementary material 1: Study protocol

Study design and setting

We conducted a randomized controlled trial to determine whether HPV self-sampling among non-attenders to Slovenian cervical screening program ZORA could increase participation. Eligible women were randomly selected from National Cervical Cancer Screening Registry (the ZORA registry) and randomly allocated to opt-in (I1), opt-out (I2) and comparison (P) study groups. Three different self-sampling devices were used in the opt-out study group, which had three parallel arms regarding the type of self-sampling device. All participants had free access to cytology screening with their personal gynaecologist (PG) during the study, who is also a provider of regular cervical screening in Slovenia, while the women in the opt-in and opt-out intervention groups also had free access to human papillomavirus (HPV) self-sampling at home. Figure 1 presents the study design and main results.

In Slovenia, cervical cancer screening is covered by the Health Insurance Institute of Republic of Slovenia. With free access to their PG, women do not need a special invitation to schedule a screening appointment. If a woman fails to make an appointment on time, her PG should invite her to make one, according to the rules on carrying out preventive healthcare at the primary healthcare level. In case she does not respond, the PG should send her another invitation. The ZORA registry serves as a final supervisor of screening attendance. Where a woman has not had cytology result registered for four years, she receives a status of non-attender and is as such eligible for central invitation, which is sent to her in the fifth year by the Institute of Oncology Ljubljana (IOL). The central invitation package includes an invitation letter, an informational leaflet about the ZORA programme and detailed information about gynaecologists working in the region of the woman's place of residence. Women are encouraged to call their PG to make a screening appointment. Women who do not have a PG are encouraged to call one of the gynaecologists from the national list. If a cytology result is not registered after the first central invitation, a second central invitation is sent one to two years after the first one. If the woman fails to respond, she receives the status of "final non-attender" and is not invited by the central coordination office again until a new cytology result is registered. However, GPs should still send regular invitations to such women. Women with pathological screening results or with histologically confirmed cervical lesions are managed according to national guidelines for the management of women with precancerous lesions and cervical cancer. Women with atypical squamous cells of undetermined significance (ASC-US) or low grade squamous intraepithelial lesion (LSIL) are invited back to their PG after six months for a triage HPV test and repeat cytology. In women with LSIL younger than 35 years, only repeat cytology is performed. Women with other pathological screening results are referred to colposcopy. Following treatment of precancerous lesion, a control follow-up cytology is recommended six months later and both HPV testing and a repeat cytology 12 months after treatment. If both tests are negative, the woman is invited back 24 months after treatment for another HPV test and cytology. If both tests are again negative, women can return to a three-year screening interval. All PGs in Slovenia are involved in screening. Screening and diagnostic samples are evaluated by certified laboratories (nine for cytology, nine for pathology and two for HPV testing).

The study was coordinated by the National Cervical Cancer Screening Programme and the ZORA registry, located at the Epidemiology and

Cancer Registry Department at the IOL. Study participants were randomly selected based on eligibility criteria from the ZORA registry ten times in 2015 and were all sent an invitation within a month of being selected. The first invitation letters were sent on 6 January 2015 and the last on 1 December 2015. According to the Healthcare Databases Act, all cervical cytology results in Slovenia are recorded in the ZORA registry since 2003, all cervical histopathological reports and all hysterectomies since 2004, and all HPV test results (both triage and test of cure) since 2010. Bethesda classification has been used to report cervical cytology since 2011 and World Health Organisation classification for histology since 2015, with obligate information on CIN 2 and CIN 3 in high-grade squamous intraepithelial lesions (HSIL). The ZORA registry has an online connection with the central population registry of the Republic of Slovenia and the registry for spatial units. The ZORA registry exchanges data yearly with the Cancer Registry of Republic of Slovenia. Great care is taken to ensure the completeness and correctness of data in the ZORA registry. Regular checks for missing or incorrect data in the reports and missing reports are carried out.

All study correspondence with participants, including distribution of self-sampling kits, was carried out from the study centre at the IOL, whereby a telephone line was also made available for additional questions and rescheduling further examination appointments for women following a positive HPV test result. Women with a positive HPV test performed on self-collected samples were invited to colposcopy clinics at the regional hospitals Maribor University Medical Centre or Celje General Hospital. The researchers who participated in the study were all trained professionals involved in the regular screening process and involved within ZORA programme as epidemiologists, gynaecologists, cytotechnologists, cytopathologists, pathologists or professionals involved in HPV testing.

Eligibility

Women were eligible for the study if they were aged 30–64, had no registered cytology result in the ZORA registry for at least four years and had permanent residence in the Celje or Maribor region as recorded in the Central Population Registry of the Republic of Slovenia. Women with a hysterectomy recorded in the ZORA registry were not eligible. All randomly selected eligible women were enrolled to the study and were sent an invitation letter. Some enrolled women were later excluded from further involvement in the study for various reasons, such as returned mail due to unknown address, rejection of participation in the study, death during the study, pregnancy, never having had an intimate relationship (virgins), living abroad or new information regarding a hysterectomy prior year 2004, as explained further in the results section. Some women informed us that they had already scheduled a screening appointment or had just attended cytological screening that had not yet been registered in the ZORA registry due to a few months' delay in registration. Although some of these women were not eligible for screening due to no/very low risk of cervical cancer, they were all included in the main intention-to-screen analysis. Some of these women also performed an HPV test on self-collected samples or attended a screening visit with a gynaecologist.

Study groups

There were three study groups in our study. Women in the opt-in study group (I1) were sent an initial invitation letter, in which they were informed that they were late for screening and invited to order an HPV self-sampling kit or make an appointment with their PG for cytological screening. Women in opt-out study group I2 were informed that they were late for screening and that they would be sent a HPV self-sampling kit within two weeks if they do opt-out. Women in the comparison group (P) were sent a standardised central invitation package used in the screening programme. As in the regular screening programme, they were informed that they were late for screening and invited to call their PG (or any gynaecologist from the attached list if they did not have one) to make a screening appointment. The same leaflet with general information about HPV self-sampling was attached to the letter for both intervention groups and the same informational leaflet about the ZORA programme and the importance of cytological screening was attached to the invitational letter for the opt-in and control groups. A questionnaire and a prepaid envelope were attached with all invitations. Participants were invited to answer the questionnaire or to contact the central coordination office by phone or email to opt in for an HPV self-sampling kit or to inform us if they did not wish to participate in the study or were ineligible due to a previous hysterectomy or pregnancy or having had a recent cytological screening, in which case they did not need self-sampling. In the opt-in group, self-sampling kits were sent to participants a week

after ordering. In the opt-out group, self-sampling kits were sent via regular mail two weeks after the invitation letter was sent. If a woman informed us that she would prefer cytological screening with her PG, a standard central invitation was sent to her (though women could also make their own appointments without informing us). Two reminders were sent, within a month of the invitation letter, to women in opt-in who did not order a tester and were not excluded from further correspondence. A reminder was sent three weeks after the tester was sent to all women who received the self-sampling kit but did not return self-collected sample.

Tester arms

There were three tester arms in the opt-out study group (I2-Q, I2-H and I2-D), each for one of three HPV testers used in the study: women in I2-Q were sent Qvintip (Aprovix AB, Uppsala, Sweden), women in I2-H were sent HerSwab (Eve Medical, Toronto, Canada) and women in I2-D were sent Delphi Screener (Delphi Bioscience, Scherpenzeel, the Netherlands). Women in opt-in study group were sent Qvintip. All self-sampling kits were sent in original packaging as provided by the manufacturer. The envelopes contained an invitation to perform self-sampling, an identity card with request for telephone number, the self-sampling device (tester) in original packaging, user instructions translated into Slovene, a pre-paid envelope addressed to the laboratory and containers for self-collected samples with a transport plastic bag.

Participants were invited to take a cervicovaginal sample by themselves at home according to the user instructions. They were encouraged to contact the coordination office via letter, phone or email in the event of any uncertainty as to the procedure. They were asked to enclose the self-collected sample within a plastic bag and a completed identity card in the enclosed envelope and send it via regular mail to the laboratory address on the envelope. Qvintip and DelphiScreener samples and identity cards were barcoded; HerSwab testers were equipped with RFID chips. All codes were unique and paired with the unique woman identification number (WIN) electronically in the central coordination office before the self-sampling kits were sent out. On receipt of the self-collected samples at the laboratory, the identity cards were checked by the laboratory personnel and the women's identity compared to the information retrieved from the scanning barcodes or RFID chips attached to the sample. As a result of this double-check approach, we encountered no issues with participant identification during the study.

Laboratory testing

All self-collected samples were tested for the presence of 13 high-risk HPV types using the clinically validated test Hybrid Capture 2 High-Risk HPV DNA assay (HC2, Qiagen, Hilden, Germany), following the manufacturer's instructions. The cut-off value of RLU/CO = 1.00 pg/ml was used to distinguish between positive and negative HPV results. Self-collected samples were evaluated for cellularity control by visual pellet assessment after centrifugation. Self-collected samples with negative HC2 result and no visual pellet were processed further. The DNA concentration was measured and, if it was below a cut-off point of 5 ng/ul, the sample was regarded as technically inadequate. Samples taken by a practitioner were processed and analysed in the same laboratory with the same assay as self-collected samples. Cellularity control on samples taken by practitioners was not undertaken, on the grounds that all samples had been taken by experienced gynaecologists.

The cytology and histology samples from women with positive home HPV test results who attended the prescheduled further examination at one of the two regional hospitals were evaluated in hospital laboratories by experienced laboratory professionals participating in the ZORA programme by standard protocols. All other samples from these women were sent to the IOL, where they were processed, analysed and stored. The cytology and histology samples from women who attended screening or a follow-up visit with their PG were sent to one of the nine cytology or pathology laboratories participating in the regular ZORA programme and were evaluated by the same experienced laboratory professionals who participate in the programme, again by the same standard protocols.

Clinical management of women following a positive HPV test performed on self-collected samples

Letters with the results of HPV testing and further recommendations were sent to all women who performed self-sampling, generally within two weeks after the self-collected sample arrived at the laboratory. A survey for assessment of the women's experience with self-sampling was sent together with the result. Women with negative results were ad-

vised to attend regular cervical cancer screening in after three years. Women with technically inadequate results were sent a standardised central invitation and were encouraged to perform cytology screening with their PG as soon as possible. Women with positive HPV test results received an informational leaflet with additional information about the positive result. They were invited to the regional hospital for an appointment at the colposcopy clinic on a prescheduled date and hour and also received a telephone number for rescheduling. If a woman failed to make her appointment, a nurse from the central office called her by phone to arrange a new date for an examination. If a woman failed to provide a telephone number, two pre-scheduled written reminders were sent to her by regular mail.

Participants were informed about the examination protocol at the colposcopy clinic and all signed an informed consent form prior to examination. A short gynaecological history was taken prior to examination. During the examination, two cervical smears were taken. The first smear was spread on two slides with a split-sample technique for conventional cytology and conventional p16/Ki67 dual immunocytochemical staining (ICS). After that spatula and brush were put in a container with in-house liquid medium. Slides were fixated immediately with Merckofix. The second smear was taken for HPV testing, which was carried out in the same manner as the HPV triage test in ZORA programme, with a Qiagen brush that was put in the container with standard transport medium (STM). The colposcopy was then performed. In the event of an abnormal colposcopy result, a biopsy was taken from the most abnormal areas. Participants were informed about the results and any need for further management by the gynaecologist who examined them. They were managed according to cytology and HPV test and colposcopy results. If HSIL, adenocarcinoma in situ (AIS) or cervical carcinoma was discovered treatment was applied according to national guidelines.

All participants could also make an appointment with their PG at any time during the study. All gynaecologists in Slovenia were informed by way of two letters about the study and were requested to encourage women with positive HPV test on self-collected sample to attend the pre-scheduled examination at the regional hospital or, if a woman rejected examination at the hospital, to invite her for cytological screening and manage her according to national guidelines. A copy of the screening result and the results of other cytology or HPV triage tests along with a histopathology report was sent to the ZORA registry as per the screening programme protocol.

Primary and secondary outcomes

The primary outcomes were: (i) intention-to-screen response rates among all enrolled women and (ii) high-grade histology outcomes among all enrolled women and all responders according to the age of the woman, opt-in and the opt-out approach, region of residence and the level of protection resulting from previous screening history. The intention-to-screen response rate was computed as a proportion of enrolled women with a response within one year after the enrolment. The response was defined as having an HPV test performed on self-collected sample and/or cytology with PG within one year after the enrolment. The response types were classified as 'HPV self-sampling only' (type A response), 'cytology screening with a PG only' (type B response), or 'both tests' (both HPV test on self-collected sample and cytology screening with a PG, type C response). Very few women visited PG for a cytological screening after the enrolment, but only a histological sample was taken from the cervix, mostly because the changes of the cervix were already visible. These women were considered type B responders. The high-grade histological outcome was defined as the diagnosis of a CIN2+ or CIN3+ lesion within one year after enrolment in the study. High-grade histology outcomes were computed as a proportion of women with the CIN2+ or CIN3+ outcome among all the enrolled women and among the responders. Positive predictive value (PPV) of the HPV test was computed as a proportion of women with a high-grade histological outcome among all the women who attended colposcopy after a positive HPV test. Cytological and histological outcomes within one year after the enrolment were identified for all the enrolled women by linking the study database with the ZORA registry. If a woman had more than one histological outcome, the lesion with the highest grade was used in calculations.

Secondary outcomes were: (i) tester ordering in the opt-in study group, (ii) the results of the HPV test on self-collected sample, (iii) the compliance at further examinations for women with a positive HPV test, and a (iv) positive concordance of the result of the HPV testing on self-collected samples and on samples taken by a practitioner. The tester ordering was computed as a proportion of the women enrolled in the opt-in study group who opted in for a tester among all the women enrolled

in opt-in study group. The results of screening tests are presented as the proportions of positive and the proportions of technically inadequate results. The compliance was computed as the proportion of all the women who attended the examination either at a regional clinic or with their PG from all the women with a positive HPV test performed on self-collected samples. The results of the HPV testing on self-collected samples and on practitioner-obtained samples were concordant if both were positive at the cut-off value RLU/CO = 1.00 pg/ml.

Data management and statistical analyses

All relevant study data were recorded in the electronic central study database that was established within the central screening coordination office. All the data gathered at both regional hospitals were recorded on standardised forms and sent to the central coordination office, where it was entered in the electronic study database. Personalised data from the study database were linked to the ZORA registry by the unique personal identification number (PIN). For the management of correspondence, including self-sampling kits, the ADA software package was bought from the manufacturer of tester Q and was adjusted to the study needs. HPV test results were sent electronically from the laboratory information system directly to the ADA system, which was installed locally at the IOL and was accessible only to researchers from the central coordination office and the IOL cytopathology laboratory. Data were linked to the central study database via the unique WIN.

Response rates were analysed only on the intention-to-screen basis. The effect size was estimated as a relative risk ratio within the 95% confidence interval (CI). Predefined subgroup analyses of possible significant predictors for the response and histological outcomes were performed with the aim to identify common characteristics of responders and approaches with the highest response rate and high-grade lesions detection (Table 2). The univariate logistic regression was used to assess if the outcome was significantly associated with the possible predictor. The multivariate logistic regression analysis was performed for all primary and secondary outcomes to adjust the outcome for predefined predictors. The predefined predictors included in these analyses were the woman's age, the region of residence, the level of protection, and the study group. The data on predictors were obtained from the ZORA registry at the time of the random selection. The level of protection due to previous screening history was categorised as 'medium protection' if the last cytology was done 5–9 years prior to the enrolment and as 'no/low protection' if the last cytology was done 10 years or more prior to the enrolment or if the woman didn't have any cytology result in the ZORA registry. Age was used as a continuous variable in all univariate and multivariate analyses. In the subgroup analysis, women's age was described in terms of the mean and the 95% confidence intervals. Other possible predictors were described as proportions; chi-square test was used to determine if the observed difference in the distribution of proportions within subgroups was statistically significant. Additional analyses were performed to explore type-specific response rates (Figure 2), high-grade disease outcomes among responders, and high-grade disease outcomes in subgroups of women with the highest PPV for high-grade disease. All analyses were conducted with SPPS 22.0 (SPSS; Chicago, IL, USA) and the open source programme language R, using 2-tailed tests and the significance level $\alpha = 0.050$.

Sample size

Due to the piloting nature of the study, we aimed to enrol as many eligible women in the intervention groups as financial limitations permitted. A feasibility study was carried out in the year before the launch of the study to assess the technical aspects thereof and its protocol adequacy and to gain the first results that were used for the calculations in the preparation stage (unpublished data). The feasibility study indicated that the opt-out study group had 4.8-times higher tester consumption (testers sent per woman) and 3-times higher tester loss (proportion of women who failed to return a self-collected sample after the tester was sent to her) than the opt-in study group. After the discounted price for the testers and all the laboratory reagents required to conduct the study according to the protocol were negotiated with manufacturers, we aimed to send out 6,000 Qvintip kits (3,000 in the opt-in group and 3,000 in the I2-Q arm), 3,000 HerSwab kits (in the I2-H arm) and 3,000 Delphi Screener kits (in the I2-D arm). Due to the limited number of testers available and higher consumption of testers in opt-out, unequal allocation of women to the opt-in and the opt-out group was planned with an allocation ratio of 4.8:1. Based on the number of the women eligible at the time of calculation and the tester consumption assumed from the results of the feasibility study, we could allocate

SUPPLEMENTARY TABLE S1.1. Number of women each month randomised to study groups opt in (I1), opt-out (I2) and control group (P)

Month of randomisation	Study group			Total
	I1 opt-in	I2 opt-out	P control	
1	1,600	664	1000	3,264
2	1,600	664	0	2,264
3	1,600	664	0	2,264
4	0	1,140	1,600	2,740
5	1,600	1,140	0	2,740
6	1,600	1,140	0	2,740
7	1,600	1,136	0	2,736
8	1,600	1,136	0	2,736
9	1,600	1,136	0	2736
10	1,600	736	0	2,336
Total	14,400	9,556	2,600	26,556

SUPPLEMENTARY TABLE S1.2. Number of women each month randomised to opt-out (I2) tester arms

Month of randomistion	Tester			Total
	I2-H HerSwab	I2-Q Qvintip	I2-D Delphi Screener	
1	0	332	332	664
2	0	332	332	664
3	0	332	332	664
4	476	332	332	1,140
5	476	332	332	1,140
6	476	332	332	1,140
7	472	332	332	1,136
8	472	332	332	1,136
9	472	332	332	1,136
10	440	296	0	736
Total	3,284	3,284	2,988	9,556

16,000 women to the opt-in group, 3,280 women to each of the three opt-out arms, and the remaining 2,600 eligible women to the control group.

With this sample size, the study had power higher than 99% to detect a 5% difference in the intention-to-screen response rate between (a) the opt-in and opt-out approach where the same tester was used (opt-in and I2-Q), given response rates of 30% and 35% respectively, (b) the opt-in approach and comparison group, given response rates of 30% and 25% respectively, and (c) two testers (two arms in group I2), given response rates of 35% and 30% respectively. The response rate assumptions were based on the results of the pilot study. Calculations were made with the Power and Sample Size Calculation Software (version 3.1.2, 2014), using uncorrected chi-squared statistics and the significance level $\alpha = 0.05$.

Random selection and allocation

When implementing HPV self-sampling of non-participants in an organised screening programme, one should consider that the target population for the intervention constantly changes due to a constant flow of newly eligible women entering and those no longer fulfilling the eligibility criteria exiting. To ensure that the appropriate women are selected for the intervention, random selection should be made as close to the intervention as possible. After the implementation of such an intervention, one should also consider the additional workload in the laboratories due to the need to analyse the self-collected samples and the additional workload of gynaecologists due to the need to examine women following positive HPV test results. Taking all this into consideration, eligible women were randomly selected for the study from the ZORA registry and randomly allocated to study groups once per month, using a random number generator in the R open-source language (18). Randomisation was stratified by five-year age groups, screening history and region of residence. We planned to enrol women in the study for 10 months in 2015. Having calculated the sample size, we planned to enrol one-tenth of women to each intervention study group each month and 1,000 and 1,600 women to the control group in months 1 and 4. Due to the delay of HerSwab kits, women in the I2-H arm were enrolled in months 4–10 (one-seventh of the women each month). Due to vacations and sick leaves of employees in the cytology laboratory and at colposcopy clinics, the sampling and allocation for the opt-in group were not done in month 4; sampling, and allocation for the I2-D arm was not done in month 10. During the study, we also encountered an unexpected situation when the manufacturer of Delphi Screener ceased its operations and it was unclear if this tester would be available on the market again, and thus the clinical value of including this tester in the study was unclear. However, the Delphi Screener soon appeared on the market again under the Rovers Medical Devices brand (Oss, Netherlands).

In total, 26,556 women were selected from the ZORA registry and allocated to the study groups and the tester arms as shown in Supplementary Table S1 and S2. 14,400 women were randomly allocated to the opt-in study group, 9,556 to the opt-out study group and 2,600 to the control group. The women in the opt-out study group were randomly allocated to the tester arms as follows: 3,284 to the I2-Q and I2-H arms and 2,988 to the I2-D arm. The initial letter was sent to all participants within a month of the randomisation. In 2015, ten randomisations were carried out, as described in Supplementary Table S1 and S2.

Ethical considerations

The study was conducted in compliance with the Helsinki Declaration and was approved by the National Medical Ethics Committee at the Slovenian Ministry of Health (consents Nos. 155/03/13 and 136/04/14). It was financed by the Slovenian Research Agency and the Slovenian Ministry of Health (trial No. L3-5512).

The principle of informed consent was waived for the study, because it was a pilot study with the aim that the results would be directly translatable to the current screening programme. Asking women for informed consent for randomisation could have biased the main outcome, which was the response rate on the intention-to-screen basis. However, all enrolled women were sent initial invitation letters with additional information leaflets in which they were fully informed about the testing and subsequent follow-up. The women thus had to involve themselves actively through their own free will in order to perform screening. The initial invitation letter also contained a detailed explanation on how potential participants could reject participation in the study, including exclusion criteria for participation. Women were encouraged to ask and discuss additional questions with the central office by regular mail, phone or email. Women could reject further participation or inform us that they met the exclusion criteria in any phase of the study.

Supplementary material 2: Difference in type-specific intention-to-screen response rates between the I1 (opt-in) and I2 (opt-out) intervention groups

SUPPLEMENTARY TABLE 2. Type-specific intention-to-screen response rates (per 100) with 95% confidence intervals (CI) by study groups. The HPV testing on self-collected samples was not possible in the P study group (marked as not available (na))

Study group	Intention to screen response rate per 100 (%) with 95% CI			
	A-HPV only	B-Gynaecologist only	C-Both tests	Total response
I1	15.7% (15.1%–16.3%)	16.5% (15.9%–17.1%)	1.8% (1.6%–2.1%)	34.0% (33.2%–34.8%)
I2	24.4% (23.6%–25.3%)	9.6% (9.0%–10.2%)	3.6% (3.3%–4.0%)	37.7% (36.7%–38.6%)
P	na	18.4% (16.9%–19.9%)	na	18.4% (16.9%–19.9%)