A randomized, controlled trial of two strategies of offering the home-based HPV self-sampling test to non-participants in the Flemish cervical cancer screening program

A randomized, controlled trial of two strategies of offering the home-based HPV self-sampling test to non-participants in the Flemish cervical cancer screening program.

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Key words: self-sampling; human papilloma virus; Flemish cervical cancer screening program; non-responders

Abbreviations used:

PAP: Papanicolaou

GP: general practitioner

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hr HPV: high-risk human papilloma virus

PCR: Polymerase Chain Reaction

OR: Odds ratio

95% CI: 95% confidence interval

Article category: research article

Novelty and impact: We evaluated the effectiveness of two different strategies of offering an HPV-self sampling program, and compared these strategies with the standard recall letter or no intervention at all. Our results support the efficacy of a self-sampling strategy to increase participation in the Flemish screening program. Self-sampling seems particularly acceptable to postmenopausal non-responders. Future research should focus on the accuracy and performance of different self-sampling devices in specific age cohorts.

Abstract

We conducted a randomized, controlled trial to evaluate different strategies of offering an HPV-self sampling program, and compared this with two control groups.

All total of 35,354 women who did not participate in the Flemish cancer screening program were included in the study: 9,118 received a HPV self-collection brush (RIATOL qPCR HPV genotyping test (qPCR (E6/E7); 9,098 were offered the opportunity to order an HPV-selfsampling brush , 8,830 received the recall letter; 8,849 received no intervention.

Within 12 months after the mailing, 18.7% of the women who had received the brush, participated by returning a self-sample sample, while 10.6% women allocated to the opt- in group did so. 10.5% women who received the standard recall letter, had a PAP smear taken within a period of 12 months; while 8% women did so without receiving an intervention at all. Participation in postmenopausal women was higher than in women younger than 50 in both self-sampling arms. Screening by means of the self-sample kit increased by age, contradictory when screening is performed by a PAP smear. Of those testing hrHPV positive (9.5%), 88.9% attended for follow up cytology. The mean DNA concentration, found in the self-sampler, decreased by age, causing a higher number of inconclusive results.

Our results support the efficacy of a self-sampling strategy to increase participation in the Flemish screening program. Self-sampling seems particularly acceptable to postmenopausal non-responders.

Future research should focus on the performance of different self-sampling devices in post-menopausal women as low DNA concentrations exponentially increased over age.

Introduction

Cervical cancer is a highly preventable disease, consequently screening is recommended by the European Union[1]. The burden of cervical cancer in Belgium ranks at the lower half among the member states in the European Union [2]. The crude incidence rate is 11.5 cases per 100 000 women-years for 2014 (http://www.kankerregister.org/default.aspx?lang=EN) . Prior to 2013, screening was essentially opportunistic in Belgium, which means that PAP smears are taken at the spontaneous initiative of the woman, her gynaecologist or her general practitioner (GP). In June 2013, an organised screening program, promoting one cervical smear every 3 years for women aged between 25 and 64 years, was set up, however only in the Flemish Region. The Flemish Cervical Screening Program aims to reduce the number of women developing cervical cancer by detecting pre-malignant changes in the uterine cervix. The programme is financed by the Flemish government. The screening program uses a centralized invitation procedure; all invitation letters are sent out by the Centre of Cancer Detection. The target population is identified from the central population registry of Flanders.

All cervical cytology results (both organized and opportunistic), histological diagnoses from cervical biopsies as well as all other pathology specimens are recorded by the national cancer registry. The reporting to the Belgian Cancer Registry is mandatory and ensures a 95% completeness of records in the registries. The cyto-histopathology registry is completed with administrative data from health insurance companies (e.g. hysterectomy). The National Social Security Number, an unique 11-digit personal identification number, is used to cross-link the population registry with the data collected by the Belgian Cancer Registry. Women with a recent spontaneous smear or those who have had a hysterectomy are excluded to receive an invitation letter, in addition to women who actively opted out of the screening program. The standard invitation letter encourages women to book an appointment with a GP or gynecologist of choice to have a PAP smear taken. Women who do not respond to this recall letter, will receive a subsequent one after 36 months. Currently, the coverage of Flemish screening for cervical cancer remains suboptimal around 61%. Participation is higher among younger women, dropping to below 50% in women older than 55 years. 47% of all invasive cervical cancers are found among un-screened women (https://baarmoederhalskanker.bevolkingsonderzoek.be/professionelen/literatuur). Negative physical and emotional experiences of screening, competing priorities and practical barriers are, among others, reasons for non-attendance[3].

Infection with human papillomavirus has been identified as a necessary cause of invasive cervical cancer [4]. Offering high risk human papillomavirus (hrHPV) DNA testing on self-taken samples (self-sampling) to the non-attendees of the routine screening helps to overcome practical and emotional barriers to screening, by allowing women to take a sample in the privacy of their home, without undergoing a gynecological examination. Self-sampling has therefor the potential to increase attendance among women who are not reached by the routine screening program [5]. However, high compliance to follow up recommendations among hrHPV positive women is necessary to achieve the wanted benefit from self-sampling. An meta-analysis comparing self-collected HPV test samples,

using a validated Polymerase Chain Reaction (PCR-based HPV) to physician collected samples found both methods to have a similar sensitivity [6].

Self-sampling kits may be mailed to non-attenders, although this "opt- out approach" induces waste of distributed and unused kits. An alternative approach is to have non-attenders actively "opt- in". This approach consists of offering the possibility to order the self-sampling kits if interested after receiving an invitation. The opt-in strategy calls for an additional effort from women to verify their willingness to participate and is therefore prone to a lower attendance [7-9].

The aim of the study was to evaluate the effectiveness of two different strategies of offering HPV-self sampling to women who do not participate in the Flemish cancer screening program, comparted with the standard recall letter or no intervention at all. We also determined the proportion of women with a positive HPV self-sampling test undergoing appropriate follow up.

Materials and methods

Study population

Our study population was limited to non-responders. A non-responder was defined as a woman, invited by the Centre of Cancer Detection at least once, aged 30-64 years, eligible for screening, without any cytology, histology or pathological result recorded since 2008, residing in the study region.

Invitees were restricted to women who had not participated in the cervical cancer screening since 2008, as the Belgian Cancer Registry does not comprise older cervical cytology data. Women who are younger than 30 years were not included due to the decreased positive predictive value of HPV DNA tests in younger women.

Being pregnant, having had a hysterectomy, a recent PAP smear or cervical cancer were exclusion criteria. As the study is embedded in the Flemish Cervical Cancer Screening program, women not eligible for an invitation for the screening program (see above) were excluded. Pregnancy was registered based on self-report (exclusion criterion after sending the letters) .

The study region consisted of 13 Flemish municipalities. Gynecologists and GP's in the region were informed about the study and the importance of a follow up examination in case of a hrHPV positive sample.

Study design

The study was a parallel, randomized, controlled trial with four arms (two intervention arms and two control arms). Eligible women were randomly assigned by a computer-generated randomized schedule following simple randomization procedures in a 1:1:1:1 ratio. Figure 1 shows the study design and flow diagram.

1. Women randomized to the first group were sent a kit to perform a self-sample for HPV testing. The kit consisted of an recommendation letter, an informative flyer, and a self-sampling brush (Qvintip, Aprovix AB) with an instruction letter explaining step by step how to collect a sample and a pre-stamped return envelope. The recommendation letter informed

about the possible results of the self-sampler, and included an informed consent to perform the test and have their physician contacted in case of positivity. The leaflet provided information about HPV, cervical cancer and possible reasons for ineligibility.

2. Women randomized to the opt-in group were sent a letter offering them the opportunity to order a HPV self-sampling kit, either by phone, mail or the study website. The letter was accompanied by the same informative flyer of the first group.

All material was sent in Dutch. A French and English translation of the material was available on the study website. In addition, the website displayed an animated instruction video how to collect a sample.

After 4 months, the women, allocated to group 1 and 2, who had not participated or withdrawn, were sent a reminder letter.

3. The women allocated to the third group were sent the regular recall letter of the Flemish screening program, inviting them to have a PAP smear taken, by a GP or gynecologist of choice (first control group).
4. The women randomized to the fourth group did not undergo an intervention (second control group).

Analyses of samples

Dry self-collected Qvintip samples were sent at ambient temperatures to AML (Sonic Healthcare, part of the National Reference Centre HPV, Belgium), the central laboratory were all HPV analysis are performed.

Upon arrival in the laboratory, Qvintip brush head was transferred into a 20 mL Hologic ThinPrep PreservCyt vial, vortexed and stored at room temperature (15-30°C) until further analysis. Processing was done in an automated manner.

The RIATOL qPCR HPV genotyping test (qPCR (E6/E7) is an iso-certified (ISI 15189) test and clinically validated according to both the Meier criteria and the Valgent criteria. The RIATOL qPCR HPV genotyping test (qPCR (E6/E7) is independently recognized as a valid HPV test for primary HPV screening as described by Arbyn et al. (2015)[10-12]. Briefly, prior to testing, the LBC vial was vortexed for 15-20 seconds and placed in the Sample Transfer System STS (Hologic Inc) and representative aliquots of 2ml were transferred in a deep-well plate. Fully automated DNA extraction was done exploiting standard boom extraction with magnetic beads using the Genfind® DNA extraction kit (Hologic). Thereafter, sample DNA was amplified on the LightCycler 480 (Roche). The presence of different HPV geno-types was determined using a series of TaqMan-based real-time PCR's targeting type specific sequences of viral genes. The RIATOL qPCR HPV genotyping test (qPCR (E6/E7) test detected 14 high-risk HPV types: HPV16 E7, 18 E7, 31 E6, 33 E6, 35 E6, 39 E7, 45 E7, 51 E6, 52 E7, 56 E7, 58 E6, 59 E7, 66 E6, 68 E7. Cellularity control was performed by amplification of the beta-globin gene. A standard curve was constructed from serial dilutions of known quantities a synthetic gene construct (g-block IDT) containing the beta-globine target sequence. Based on the beta-globin standard curve, DNA concentration (ng/µl) was

determined. Samples with a DNA concentration below 0,12 ng/µl were considered as invalid and reported as inconclusive. Below this cut-off, consistency is not guaranteed. Results were reported as hrHPV negative, hrHPV positive or inconclusive. An inconclusive result included no or insufficient material/cells for analysis.

Follow up of HPV tested women

All women tested by use of self-sampling, and their GP, received a written test result by ordinary mail. Women with a hrHPV negative result were recommended to participate in the next round in the screening program. Women with a hrHPV positive result were urged to have a PAP smear taken. Data from follow up examinations were collected through mailings and telephone calls to the women or the referent physicians. Missing PAP smear results were collected from the Belgian Cancer Registry.

Women with an inconclusive result were advised to the PAP smear taken, as the self-test does not seem to be appropriate for them.

Ethical approval

The study was approved by the Ethical Committee of the University Hospital Leuven. From the two self-sampling groups, signed informed consents were acquired from all participants.

Statistical analysis

An intention to treat analysis was adopted. Letters and kits returned to sender, were analysed as randomised. Women who were excusable by self-reporting a hysterectomy, being pregnant or having a PAP smear taken, were equally kept in the intention-to-treat analysis. The primary outcome was participation, defined as having a self-sample analyzed and/or having a cervical smear taken by a physician within 12 months after the mailing. Odds ratios (OR) and 95% confidence intervals for participation were estimated using logistic regression.

The secondary outcome, which was applicable only to the women in both self-sampling arms with a positive HPV test, was compliance with the follow up PAP smear. Subgroup analyses by age were performed.

All statistical analyses were done in SPSS.

Results

Participation

A total of 35,354 women were included in the study: 9,118 received a HPV self-collection kit; 9,098 were allocated to the opt-in option, 8,830 received the recall letter and 8,849 received no intervention at all. In total 308 women (181 for group 1 and 128 for group 2) answered they were not suitable to participate. Most of them (208) reported they had a hysterectomy; 7 of them reported they were pregnant.

Within 12 months after the mailing, 1,707 women (18.7%) in the self-sampling group had participated by returning a self-sample sample, while 965 women (10.6%) allocated to the opt-in group did so. When participation by means of having a PAP smear taken was included, participation augmented to 25.8% and 18.7%. 931 (10.5%) women who received the recall letter, had a PAP smear taken within a period of 12 months; while 709 (8%) women did so without receiving an intervention at all (table 1).

Participation (by having a PAP smear taken) dropped with increasing age in both the recall letter group as in the group with any intervention. In contrary, in both self-sampling arms, when participation by self-sampling exclusively was considered, participation was lowest among women younger than 45 years old. Participation in postmenopausal women was higher than in women younger than 50 in both self-sampling arms (20.1% versus 17% for group 1; 11.8% versus 9% for group 2). The total participation (either by self-sampler or PAP smear taken) was the highest among women aged 30-39y (table 1).

1252 women of the 'opt-in' group ordered a self-sampling kit; however 287 (22.8% of those who ordered) did not return a sample.

Table 2 shows the odd ratios of participation in the two self-sampling groups and the recall letter group in comparison to the group absent of an intervention, and the odd ratios of participation in the two self-sampling groups in comparison to the group who received the recall letter. Women mailed directly the self-sampling kits were 3.2 (95% CI: 3.0-3.5) times more likely to be screened (either by self-sampling or PAP smear) compared to women lacking an intervention. The youngest women were twice as likely to be screened (OR: 2.1; 95% CI: 1.8-2.5) while the eldest women were then six time as likely to screen (OR: 6.4; 95%cI: 5.0-8.0) (data not shown).

Women who received the recall letter urging them to have a PAP smear taken were 1.3 (95% CI 1.2.-1.4) times more likely to be screened compared to women lacking an intervention.

Women receiving the option to receive a self-sampling kits were 1.8 (95% CI 1.7-1.9) times more likely to be screened (either by self-sampling or PAP smear) compared to women receiving the recall letter (table 3). The eldest women (60-64 years) were 2.7 (95% CI: 2.2-2.4) times more likely to be screened (data not shown).

Results of the self-samples and HPV positivity rates

The overall hrHPV prevalence was 9.5% in the two self-sampling groups combined; hrHPV prevalence was 12.6% at age 30-34 years, and 6.6% at age 60-64 years. The hrHPV prevalence was 10.5% in the first group and 7.7% in the opt- in group ($p<0.05$).

Overall, in 11.8% of the women no conclusive result could be determined. In post-menopausal women, the prevalence of an inconclusive result increased dramatically to 20.2% by women aged 60-64 years (Figure 2).

Among the women tested positive, HPV type 16 (found in 27.2% of the positive samples) was most frequently identified, followed by types HPV 31, 56, 51, 66 and 68 (respectively: 17.7%, 14.4%, 10.7%,

9.9% and 9.3% identified in the positive samples). 21% of all hrHPV positive women were infected with at least two hrHPV types.

Consistent with the increasing proportion of inconclusive results by age, the mean DNA concentration, found in the self-sampler, decreased by age (Figure 3).

Follow-up of hrHPV positive women.

Out of the 243 women with hrHPV positive results, 216 (88.9%) attended a scheduled appointment with their GP or gynecologist to have a PAP smear taken. Compliance with a follow up PAP smear was lowest in the youngest age group (79.4% in women aged 30-34 years) (data not shown). In 56.9% no atypical cells were found (table 3).

In 10.2 % of the PAP smears done after a hrHPV result, a high grade lesion was detected.

In 22 women a CIN2+ lesion was found, leading to a CIN2+ detection rate of 8.2 (per 1,000 women screened).

Discussion

The Flemish cervical cancer screening is confronted by a suboptimal participation rate. Innovative strategies to target not-responders and to increase participation are needed. The purpose of the study was to assess the effects of two methods of offering self-sampling tests to the non-attendees of the routine cervical cancer screening program in Flanders, compared to the standard recall letter and no intervention at all. Self-sampling has the potential to overcome practical and perceived barriers to cervical cancer screening. A meta-analysis of seven included studies estimated that, on average 97% of women (95%CI 95-98%) found self-sampling to be generally acceptable. Reported reasons for disliking self-sampling were uncertainty about self-sampling correctly, pain or physically uncomfortable, and anxiety [13].

In our study, we showed that directly mailing a self-sample to non-responders of the regular screening program significantly increases the response rate compared to a recall letter to have a PAP smear taken by a physician. A systematic review and meta-analysis to evaluate the participation after offering a device for self-sampling, showed that the pooled participation, in an intention-to-treat analysis was 23.6% (95% CI: 20.2-27.3%) when the self-sampling kit was mailed directly [14]. In our study, 18.7% of the women allocated to the first group participated. The meta-analysis found a pooled participation of 14.4% (95% CI: 8.0-21.4) when women had to opt-in to receive a self-sampling kit[14]. In our study 10.6% of the women allocated to that arm, did so.

We observed that 22.8% of the women who ordered the self-sampling kit did not return a sample. A Copenhagen-based, opt-in study reported a loss of self-sampling kits of 11%. Using written communication, literacy and health literacy limitations may have impeded response to this study.

hr HPV prevalence was 9.5%. This is equal to the prevalence (9.58%) found in 16,549 preventive PAP smears taken from Flemish women aged 30-64 years, during the study period (unpublished data obtained from NRC HPV Belgium). In a Dutch study among non-attenders 10.3% of the women tested positive [15]. In a responder screening population of the Netherlands 10.0% of the self-samples tested

positive[16]. The prevalence of the hrHPV positivity will be influenced by the specificity of the combination of test method and sampling method. This warrants for development of guidelines and criteria for HPV tests.Significantly more women of the first group tested positive than women in women in the opt- in group, suggesting that women with a higher risk for cervical cancer are more easily persuaded to participate when the self-sample is directly mailed. There was no differences in histological findings between the two self-sampling arms, however conclusions should be drawn with great caution, as the sample size of histo- cytopathological results was small.

Loss in follow-up among hrHPV-positive women must be minimized to maximize the wanted benefit from self-sampling. In our study, 88.9% attended a follow up PAP smear. The high compliance rate may have been partly induced by the phone calls and/or mailings to the women and their doctors to collect follow up data. Our results were however, comparable to the adherence achieved in a Dutch and a French study (respectively 89.1% and 90.9%) [17,18]. While in a Norwegian trial, 94.1% of women tested positive attended follow up[19]. In a Swiss study, there were 5.05% (95% CI: 3.1–8.1) women with a positive screen who did not attend triage and colposcopy[20]. In an Australian study 75.7% of the hrHPV positive women had appropriate clinical follow-up [21]. In a French study targeting a low-income population, only 41% of the women who tested positive for hrHPV had a follow up PAP smear taken [22]. A trial in a English population demonstrated a compliance for follow cytology of 59% [23].

Noteworthy, screening by means of the self-sample kit increased by age in our study, with the highest rate seen in the oldest age groups contradictory to the trend seen when screening is performed by a PAP smear. Few studies have focused on the barriers and motivators of older women concerning cervical cancer screening. Older women may perceive cervical screening to be less relevant to them than younger women[24]. Signs of cervical abnormalities from menstruation and sexual activity may no longer be present as cues to attend screening. Women become more conscious about showing their body as they age, this may add to embarrassment and deter them from screening[24]. Unfortunately, we found low DNA percentages exponentially increasing over age to more than 20% in the age-group 60-64 years. A German study comparing the performance of the Qvintip with an another self-sampling device, the Evalyn Brush (Rovers, Medical Devices) found similar hrHPV detection rates, however the observed mean DNA concentration was found to be higher with the Evalyn samples than of the Qvintip samples (mean concentration: 23.8; 95% CI 23.2-24.4) [25]. No stratification according to age group was performed. To our knowledge, our study was the first to demonstrate an decrease in detected DNA concentration according to age. As shown in our previous study, using Evalyn brush, this age dependency could not be detected (abstract IPV 2017). Further evaluation is ongoing within the VALHUDES trial. In-depth discussion will be based on the outcome of this study[26].

Future research should focus on the accuracy and performance of different self-sampling devices in combination with specific HPV-tests and in specific age cohorts.

Strengths and limitations

As the study was embedded directly into the screening program, the results are well applicable when considering the feasibility of self-sampling in the Flemish cancer screening program. The fact that the studied women were non-attendees, who had resisted multiple previous opportunities to be screened, is one of the strengths of the study. We defined a non-attendee as a woman with no registered Pap smear since 2008. All smears taken in Flanders, both in the organized screening program as opportunistic were taken into account.

However, our study has some limitations that need to be addressed. Invitees were restricted to women who have not participated in the cervical cancer screening since 2008, as the Belgian Cancer Registry does not comprise older cervical cytology data. Consequently, we were not able to identify women who had a PAP test prior to 2008 and could not perform a sensitivity analysis between truly never screened and under screened. It is a limitation in our study that we used only one type of sampling device. A Dutch study demonstrated a higher participation rate among brushbased self-sampling device group was than in a lavage based group; while a Finnish study found the differences in total attendance not significant [27 28]. The women in the control did not receive a reminder letter because we wanted to compare the effect of the self-sampling kit with the standard procedure within the screening program. We did not collect the histological results of the control arms. Finally, as both the interventions propose a HPV test while both the controls propose a Pap test, we cannot rule out the possibility that there is an additional effect of the new test per se and not of the sampling method.

In conclusion, our results support the efficacy of a self-sampling strategy to increase participation in the Flemish screening program. Self-sampling seems particularly acceptable to postmenopausal non-responders. However, future research should focus on the performance of different self-sampling devices in post-menopausal women as low DNA percentages exponentially increased over age.

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Conflict of interest

IB, DVB and JB are employed by AML, a commercial lab performing cytology and HPV testing.

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Table 1. Participation by intervention, stratified by age group with 12 months after mailing

	Mail to all					Opt in group					Recall letter			No intervention		
		Attended by					Attended by									
	Invited N	Self-sampling N	%	PAP smear	Total Partici-Pation* %	Invited N	Self-sampling N	%	PAP smear	Total Partici-Pation* %	Invited N	Attended N	%	Invited N	Attended N	%
Overall	9118	1707	18.7	649	25.8	9098	965	10.6	740	18.7	8830	931	10.5	8849	709	8
Subgroups by age category																
30-34y	913	140	15.3	156	32.4	906	69	7.6	127	21.6	952	162	17.0	991	151	15.2
35-39y	1110	166	15.0	132	26.8	1069	94	8.8	147	22.5	972	161	16.6	1038	136	13.1
40-44y	933	164	17.6	83	26.5	946	89	9.4	115	21.6	974	144	14.8	948	100	10.5
45-49y	1093	220	20.1	87	28.1	1084	110	10.1	111	20.4	1091	141	12.9	1059	91	8.6
50-54y	1219	235	19.3	72	25.2	1253	154	12.3	82	18.8	1233	105	8.5	1237	88	7.1
55-59y	1511	308	20.4	56	24.1	1510	181	12.0	73	16.8	1452	99	6.8	1472	67	4.6
60-64y	2339	474	20.3	63	23.0	2330	268	11.5	85	15.2	2156	119	5.5	2104	76	3.6

*overall participation by returning either a self-sample kit or by having a PAP smear taken

Table 2. Odds ratio of participation in the two self-sampling groups and the recall letter group in comparison to the group absent of an intervention and in comparison to the group who received the recall letter.

	Mail to all group OR (95%CI)	Opt in group OR (95%CI)	Recall letter OR (95%CI)	No intervention OR (95%CI)
Comparison to the group absent of an intervention				
Self-sampling samples	2.3 (2.2- 2.5)	1.3 (1.2- 1.5)	/	1.00
PAP smears taken into account	3.2 (3.0- 3.5)	2.3 (2.2- 2.5)	1.3 (1.2- 1.4)	1.00
Comparison to the group who received the recall letter				/
Self-sampling samples	1.78 (1.65-1.91)	1.0 (0.9- 1.1)	1.00	/
PAP smears taken into account	2.45 (2.29-2.63)	1.8 (1.7- 1.9)	1.00	/

Table 3: Cytological results of the PAP smears taken with women positive for HPV

Cytological diagnosis	N	%
NLIM	123	56.9%
ASC-US	33	15.3%
ASC-H	7	3.2%
LSIL	30	13.9%
HSIL	22	10.2%
AGC	0	/
Insufficient quality	1	0.5%
Total number of PAP smears	216	100

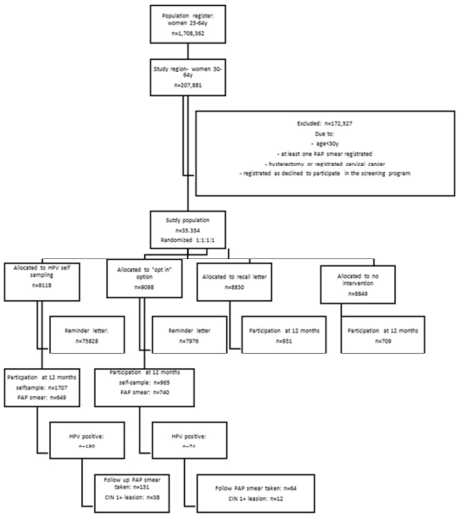
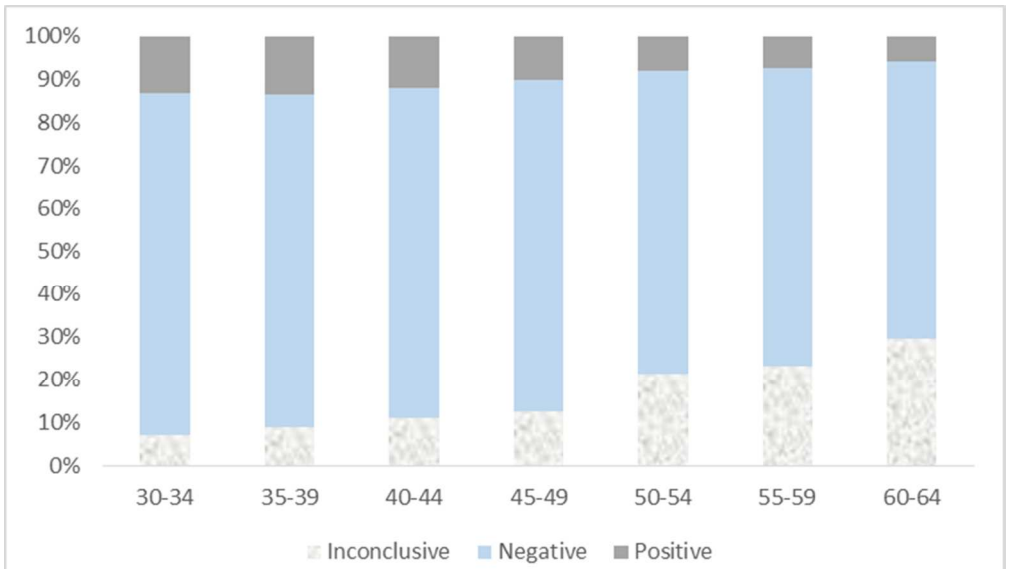


Figure 1: Flow chart of the study

338x190mm (96 x 96 DPI)



igure 2. Results of the self-samples, stratified by age group

338x190mm (96 x 96 DPI)

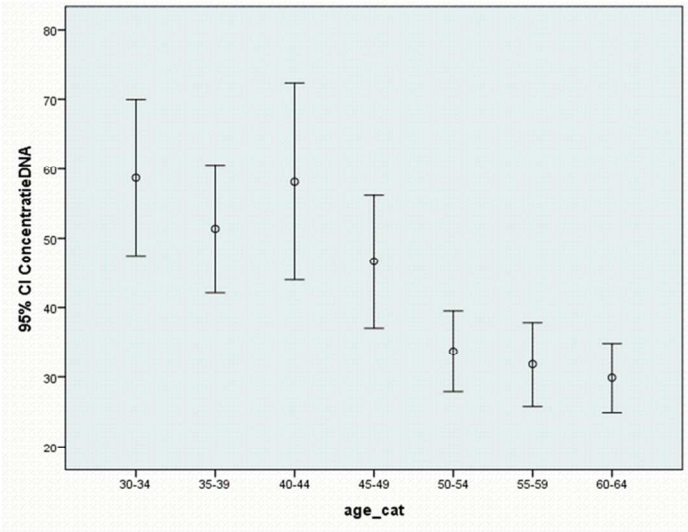


Figure 3: Mean DNA concentration, found in the self-sample, stratified by age group.

338x190mm (96 x 96 DPI)