Research Article

Cancer Prevention Research

Implementation of HPV-based Cervical Cancer Screening Combined with Self-sampling Using a Midwifery Network Across Rural Greece: The GRECOSELF Study

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Abstract

Self-sampling for human papillomavirus (HPV) testing is an alternative to physician sampling particularly for cervical cancer screening nonattenders. The GRECOSELF study is a nationwide observational cross-sectional study aiming to suggest a way to implement HPV-DNA testing in conjunction with self-sampling for cervical cancer screening in Greece, utilizing a midwifery network. Women residing in remote areas of Greece were approached by midwives, of a nationwide network, and were provided with a self-collection kit (dry swab) for cervicovaginal sampling and asked to answer a questionnaire about their cervical cancer screening history. Each sample was tested for high-risk (hr) HPV with the Cobas HPV test. HrHPV-Positive women were referred to undergo colposcopy and, if needed, treatment according to colposcopy/biopsy results. Between May 2016 and November 2018, 13,111 women were recruited. Of these, 12,787 women gave valid answers in the study questionnaire and had valid HPV-DNA results; hrHPV prevalence was 8.3%; high-grade cervical/vaginal disease or cancer prevalence was 0.6%. HrHPV positivity rate decreased with age from 20.7% for women aged 25–29 years to 5.1% for women aged 50–60 years. Positive predictive value for hrHPV testing and for HPV16/18 genotyping ranged from 5.0% to 11.6% and from 11.8% to 27.0%, respectively, in different age groups. Compliance to colposcopy referral rate ranged from 68.6% (for women 25–29) to 76.3% (for women 40–49). For women residing in remote areas of Greece, the detection of hrHPV DNA with the Cobas HPV test, on self-collected cervicovaginal samples using dry cotton swabs, which are provided by visiting midwives, is a promising method for cervical cancer secondary prevention.

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Introduction

Cervical infection by high-risk human papillomaviruses (hrHPV) is considered to be the primary cause for the development of cervical cancer (1). This has led to the development of innovative molecular HPV–based screening tests, which are being incorporated in cervical cancer screening programs worldwide, either alone or in combination with cytology, the traditional morphologic screening test (2, 3). HPV DNA testing in primary cervical cancer screening has proven to offer better long-term protection against cervical cancer than cytology (4, 5).

The advent of HPV-based molecular screening modalities has provided new possibilities for cervical cancer screening, including methods for self-collected cervicovaginal samples. Self-sampling can be effective for women who, for various reasons, do not faithfully follow cervical cancer screening recommendations (6).

In Greece, effective secondary cervical cancer prevention remains an unmet need, because cervical cancer screening is opportunistic rather than organized, resulting in only a third of the female population reporting being screened on a regular basis (7). With this in mind, we launched GRECOSELF, the GREek CObas SELF-sampling study, a multicenter observational cross-sectional study aiming to suggest a way to implement HPV-DNA testing in conjunction with self-sampling, for women residing in remote areas of Greece, including mountainous villages and islands, utilizing a midwifery network.

GRECOSELF is the largest molecular epidemiology study designed and carried out in Greece. The study utilized a nationwide midwifery network to provide self-sampling for HPV DNA testing to more than 13,000 women residing in rural areas of Greece. The primary objective of GRECOSELF was to explore the implementation of primary hrHPV DNA testing with HPV 16/18 genotyping on self-collected cervicovaginal samples and compare this cervical cancer screening strategy to current screening practices in real life. The study hypothesis was that self-sampling combined with hrHPV DNA testing and integrated HPV16/18 genotyping could have a significantly better effect on timely cervical precancer detection than current pragmatic opportunistic screening practices in Greece.

Figure 1.
Map of Greece showing the different prefectures of Greece from the remote areas of which women had been recruited for the GRECOSELF study (gray color).

Materials and Methods

Setting, patient recruitment, and sample collection

The GRECOSELF study is a nationwide observational cross-sectional study on HPV primary cervical cancer screening on self-collected cervicovaginal samples tested by the Cobas HPV test, (Roche Molecular Systems). Figure 1 shows the Prefectures of Greece from remote areas of which women were recruited for the study. Overall, 39 of the 51 (76.5%) prefectures of Greece were included. Recruitment was based on the usage of a nationwide midwifery network. This network of public sector midwives, which was formed specifically for this study, came at no extra cost because cervical cancer screening is part of their role as public health care providers. Prior to the recruitment phase, the study group initially approached midwives by contacting representatives from regional midwifery associations. Then, the group organized meetings with the members of these associations in different regions of the country during which the study design and objectives were presented. Midwives who were willing to participate were provided with a number of self-sampling kits

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Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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Cancer Prev Res 2019;12:701–10

doi: 10.1158/1940-6207.CAPR-19-0192

and instructions commensurate to the population that they cover in their practice. The study coordinators communicated with the members of the network through social media and through face-to-face meetings that were carried out frequently during the study in different cities throughout the country.

According to the study design, nonpregnant women between 25 and 60 years old who resided in rural areas of Greece were contacted by midwives through public announcement in their place of residence, and, after giving their written informed consent, were provided with a dry swab (Roche Molecular Systems) along with the necessary sampling instructions. Each participant privately collected the cervicovaginal specimen on the dry swab, either alone or under midwife supervision, mainly in a primary health care facility (Health center, General Practitioner's office) or at home; the woman inserted the swab in the vagina, and rotated it 10 times; then, she placed the swab into a tube, capped the tube, after breaking the swab on a prespecified position, and handed the tube to the midwife. Following that, each study participant filled out a questionnaire designed to give information about her history of cervical screening participation and outcomes.

All women were required to report if they had at least one Papanicolaou smear test (Pap test) in the past or not, and if the Pap test was performed within the previous three years or before that time. If the result of this Pap test was considered abnormal, the women were then asked to report which intervention this led to, that is, excisional treatment, hysterectomy, or chemotherapy/radiotherapy.

The study was approved by the Ethics Committees of the Aristotle University of Thessaloniki (237/11.04.2016) and the Centre for Research and Technology Hellas (ETH. COM-9/09/11/2015) and conducted with permission by the Hellenic Ministry of Health in close collaboration with the Second, Third, Fourth, Fifth, Sixth, and Seventh Regional Health Administrations of Greece.

Sample storage and transportation

Midwives stored the self-collected samples for up to two weeks, and then shipped them along with the accompanying information to the Institute of Applied Biosciences–Centre for Research and Technology Hellas (INAB/CERTH) for downstream analysis. The sample and the questionnaire corresponding to each participant were given a unique barcode used for pseudonymization. All subsequent analyses from that point onwards were performed pseudonymized using the unique ID number assigned to each study participant.

To identify the optimal and easiest way to store and transport the samples for the GRECOSELF project, two different temperature conditions were compared during an initial phase of the study using the first 1,113 samples collected. Of these, 557 samples were stored at 0–4°C, whereas the subsequent 556 consecutively collected samples were stored at ambient temperature. All refrigerated samples were transported following stringent transport protocols so that the temperature would not exceed 4°C. Samples stored at ambient temperature were transported without any restrictions regarding specific conditions. Of note, all samples collected during the initial period were transported during July and August 2016; during those months ambient temperature at noon was at least 30°C. The analysis of all samples with the Cobas HPV test revealed no statistical difference in invalid HPV testing result rates between the two groups (Supplementary Data S1). On the basis of that, sample storage and transportation in ambient temperature were adopted throughout the remainder of the study.

HPV DNA testing

Dedicated personnel at the INAB/CERTH received the samples enclosed in special biological packaging (UN 3373), recorded the date, temperature, region, and barcode, and prepared the samples to be tested using the Cobas HPV test (Roche Molecular Systems). This test is designed for the identification of the 14 hrHPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) offering partial genotyping for HPV16 and 18. Therefore, it yields a positive, negative, or invalid result for three channels, namely, for HPV16, HPV18, and for the panel of 12 other hrHPVs (non 16/18). We considered a result as invalid when at least one out of the three channels yielded an invalid result. The test distinguishes a positive from a negative HPV test according to cut-off points or cycle thresholds (C_t), which have been established for each channel, using an iterative algorithm to achieve a defined clinical sensitivity for CIN2+, so that it produces the maximum estimate of specificity among the possible *C*t values that satisfy the sensitivity requirement. This condition was fulfilled using the cut-off values of C_t, 40.5, 40, and 40, for the three channels, respectively (8).

Preparation of the self-collected samples for DNA detection included the addition of 4.3 mL of Cobas PCR media (provided as a bulk reagent by Roche, 04773063001) into the sample tube, vortexing for at least 20 seconds, and the removal of the caps from the tubes. Finally, the tubes were loaded into the Cobas 4800 sample rack prior to the onset of the automated analysis with the Cobas 4800 system, according to the protocol used for physician-collected samples.

Colposcopy

Women positive for at least one hrHPV were referred for colposcopy at one of the participating academic or national health system hospitals closest to their place of residence. All colposcopies were performed by medical doctors, specialists in colposcopy and cervical pathology. If the

colposcopic findings were within normal limits (WNL; no signs of cervical intraepithelial neoplasia–CIN), the woman was scheduled for a cytologic examination in 12 months. In case of abnormal or suspicious for invasion colposcopic findings, multiple focal biopsies, and/or endocervical curettage (ECC), were taken from the abnormal area of the cervix. ECC was performed in cases with a transformation zone type III.

Histopathology

Histopathologic examinations were conducted in each of the participating hospitals by two expert pathologists specialized in cervical pathology. These specialists were aware of the colposcopy result, but not of the hrHPV DNA genotyping result, save for the fact that the women referred to colposcopy would be hrHPV positive. If the biopsy/ECC results were WNL (no signs of CIN), the woman was instructed to proceed with normal, routine screening. If the biopsy revealed low-grade CIN (CIN1) lesions, the woman was instructed to undergo reexamination after 12 months. In the case where a biopsy revealed high-grade CIN lesions (CIN2–3) or cancer (CIN2+), the patient was immediately informed of treatment options. Abnormal findings (CIN of any degree) were independently reassessed by a second specialized pathologist, and in the case of a disagreement between the two experts a third expert examined the specimen in question, so that at the end a two out of three decision could be made.

Table 1. Demographic characteristics of the study population

	Total
Eligible women	13,111 (100.0%)
Age (years)	
Mean (SD)	44.42 (9.7)
Median (range)	45 (18–75)
Ethnic origin	
White	13,111 (100%)
Postmenopausal	4,020 (30.7%)
HPV vaccinated	367 (2.8%)
Number of children	
0	1,543 (11.8%)
1–2	8,182 (62.4%)
>3	3,026 (23.1%)
Smoking history	
Nonsmoker	8,266 (63.0%)
Current smoker	4,703 (35.9%)
Ex smokers	1,715 (13.1%)
Marital status	
Single	1,249 (10.1%)
Married	10,583 (80.7%)
Widow	351 (2.7%)
Divorced	751 (5.7%)
Education	
No education	102 (0.8%)
Primary (6 y)	2,836 (21.6%)
Secondary (9–12 y)	5,679 (43.3%)
Higher	323 (2.5%)
Health care cost coverage	
Private	2,283 (17.4%)
Public insurance	9,803 (74.8%)
Private insurance	329 (2.5%)

Statistical analysis

During the GRECOSELF study design phase, we made our estimate of the required sample size of women based on the expected hrHPV DNA testing sensitivity, and the respective confidence interval (CI), as well as CIN2+ prevalence rate. The former was set at 90.0% (9); the latter, according to the largest relevant study in Greece on urban population (10), was 1.07%, hence, we assumed a prevalence rate of 0.8% for the GRECOSELF sample size calculation, because our target population would presumably have lower hrHPV prevalence than an urban population, a fact which has been shown in a former study conducted in Greece (7). By utilizing standard knowledge regarding estimators of sensitivity, and prevalence of CIN2+, we estimated the required sample size, expecting a precision of 0.35 in the 95% CI for sensitivity. The sample size was estimated to be at least 12,725.

Standard descriptive statistical analysis was used to report demographic characteristics and historical data regarding cervical cancer screening of the total population, as well as molecular, colposcopical, and histologic results. All laboratory results along with questionnaire data from participants were securely and anonymously captured in Google forms, accessed by authorized study coordinators, and finally exported in an excel database used for the study analyses. All statistical analyses were conducted with IBM SPSS v. 24.0.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was performed subject to the: Ethics Committees of the Aristotle University of Thessaloniki (237/11.04.2016) and the Centre for Research and Technology Hellas (ETH. COM-9/09-11-2015).

Informed consent

Informed consent was obtained from all individual participants included in the study.

Results

Demographic characteristics

Between May 2016 and November 2018, 13,111 women were recruited for the GRECOSELF project. The mean age was 44.4 and the majority of the recruited women were premenopausal (69.3%) and unvaccinated (97.2%) against HPV (Table 1). Most women (62.4%) had one or two children, and were married (80.7%). Almost half of the women participating (49.0%) were either current or former smokers.

Study flowchart

Overall, of the 13,111 samples tested, 267 (2.0%) yielded an invalid HPV result; therefore, 12,844 women with a valid HPV result were included in the analysis. Of these, 11,774 tested hrHPV negative and 1,070 (8.3%) women tested positive for at least one hrHPV type. A total of 148 of the 1,070 (13.8%) tested positive for HPV16 only and 41/1,070 (3.8%) for HPV18 only. A total of 773 of the 1,070 (72.2%) hrHPV-positive women were negative for HPV16/18 and positive for at least one other hrHPV types; 71/1,070 (6.6%) were positive for HPV16 and at least one other hrHPV except HPV18; 32/1,070 (3.0%) tested positive for HPV18 and at least one other hrHPV except HPV16. Finally, five women (5/1070, 0.5%) tested positive for HPV16 and HPV18 and at least one of the other hrHPVs tested (Fig. 2).

According to the study protocol, women positive for at least one hrHPV were referred to have a colposcopy. Therefore, 1,070 colposcopies needed to be performed; however, due to various reasons (patient refusal, missing report, pregnancy, woman not reached), the colposcopy report was available for 774 women (72.3%). Colposcopically guided biopsies were taken in 237 cases having an abnormal colposcopic impression revealing: negative findings in 74/237 (31.2%), CIN1 in 86/237 (36.3%), CIN2 in 28/237 (11.8%), CIN3 in 44/237 (18.6%), and vaginal intraepithelial neoplasia in 2/237 cases (0.8%). Moreover, there were 1 each case (0.4%) of adenocarcinoma in situ (AIS), 1 case of invasive adenocarcinoma (0.4%), and 1 case of squamous cell carcinoma (SCC; 0.4%). Overall, the prevalence of high-grade cervical disease or cancer (CIN2+) was 0.6% (75/12,843).

Cases of CIN2+ associated with HPV16/18 were detected in 44 women (39 associated with HPV16; three associated with HPV18; and two with HPV16/18, partially among others) while cases with CIN2+ due to infections by other hrHPVs (non 16/18) were detected in 31 women.

Cervical cancer screening history

In total, of the 12,787 women who yielded a valid HPV DNA result during the GRECOSELF study and gave valid answers in the survey conducted within the study, 653 (5.1%) reported having never had a Pap test before (Table 2). HPV DNA testing with self-sampling during the GRECOSELF project led to the detection of five cases of CIN2+ (three CIN2 and two CIN3) among these women (Table 3).

Women who reported that they had a Pap test more than three years prior to their recruitment in the GRECOSELF study (N = 2,198, 17.2%) reported that the result was abnormal in 82 cases, and that they were treated with excisional treatment in 15 cases,

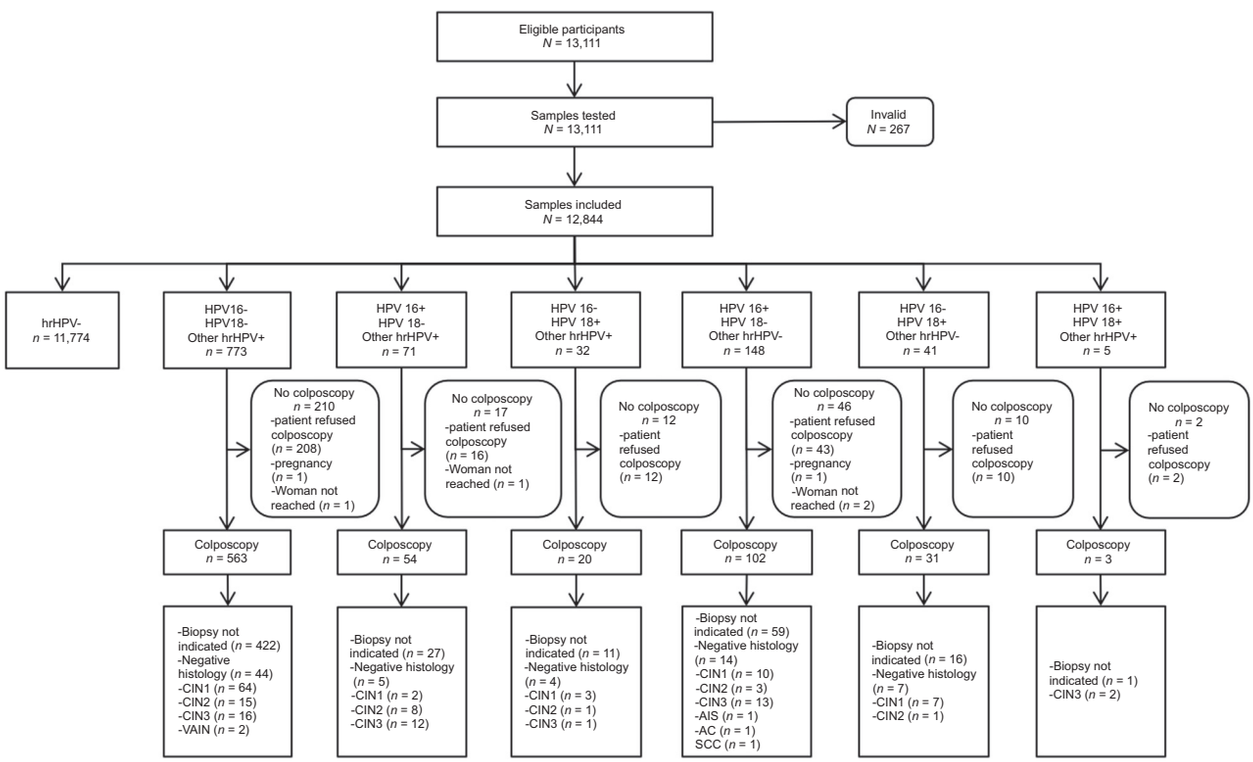

Figure 2.
Flowchart of the study.

Table 2. Cervical cancer screening history of the women participating in the study with a valid hrHPV DNA test result and valid answers in the nested survey

		HPV positive women *N*(%[a]; referred to colposcopy)					
				Colposcopic impression: abnormal (biopsy)			
Screening history	HPV negative women *N*(%[a]; not referred to colposcopy)	Colposcopy missing	Colposcopic impression: WNL (no biopsy)	Histology: WNL	Histology: CIN1	Histology: CIN2+/VaIN	Total study population *N*(%[a])
Women reporting NEVER having a Pap test	587 (89.9%)	19 (2.9%)	30 (4.6%)	7 (1.1%)	5 (0.8%)	5 (0.8%)	653 (100.0%)
Women reporting having a Pap test before the previous 3 years	2,056 (93.5%)	40 (1.8%)	61 (2.8%)	14 (0.6%)	14 (0.6%)	13 (0.6%)	2,198 (100.0%)
Women reporting having a NEGATIVE Pap test before the previous 3 years	1,797 (93.6%)	36 (1.9%)	50 (2.6%)	12 (0.6%)	12 (0.6%)	12 (0.6%)	1,919 (100.0%)
Women reporting having a POSITIVE[g] Pap test before the previous 3 years	72[b] (87.8%)	3[c] (3.6%)	5[d] (6.1%)	1[e] (1.2%)	0 (0.0%)	1[f] (1.2%)	82 (100.0%)
Women reporting having a Pap test within the previous 3 years	9,076 (91.3%)	237 (2.4%)	444 (4.5%)	53 (0.5%)	67 (0.7%)	59 (0.6%)	9,936 (100.0%)
Women reporting having a NEGATIVE Pap test within the previous 3 years	8,385 (91.6%)	214 (2.3%)	397 (4.3%)	49 (0.5%)	56 (0.6%)	49 (0.5%)	9,150 (100.0%)
Women reporting having a POSITIVE[g] Pap test within the previous 3 years	356[h] (84.6%)	15[i] (3.6%)	32[j] (7.6%)	3[k] (0.7%)	8[l] (1.9%)	7[m] (1.7%)	421 (100.0%)
Total	11,719 (91.6%)	296 (2.3%)	535 (4.2%)	74 (0.6%)	86 (0.7%)	77 (0.6%)	12,787 (100.0%)

Abbreviations: CIN1, Cervical intraepithelial neoplasia grade 1; CIN2+, Cervical intraepithelial neoplasia grade 2 or worse; HPV, Human papillomavirus; Pap test, Papanicolaou test; VaIN, Vaginal intraepithelial neoplasia; WNL, Within normal limits.

[a]All percentages are calculated per row. Numbers in columns do not sum up due to missing values (women not replied to some of the questions).

[b]Thirteen women reported being treated with excisional treatment, 17 women reported being treated with hysterectomy, and 1 woman reported being treated with radio/chemotherapy. The remaining women reported not having received any treatment or did not answer.

[c]These women reported not having received any treatment or did not answer.

[d]One woman reported being treated with excisional treatment and 2 women reported being treated with hysterectomy. The remaining women reported not having received any treatment or did not answer.

[e]This woman did not answer.

[f]This woman reported being treated with excisional treatment.

[g]Positive pap test was defined in the questionnaire as a result requiring referral to the gynecologist for further evaluation. Inflammation, metaplasia and other conditions apart from dysplastic cellular abnormalities were excluded.

[h]Eighty-two women reported being treated with excisional treatment, and 36 women reported being treated with hysterectomy. The remaining women reported not having received any treatment or did not answer.

[i]Three women reported being treated with excisional treatment. The remaining women did not answer.

[j]Five women reported being treated with excisional treatment, and 5 women reported being treated with hysterectomy. The remaining women reported not having received any treatment or did not answer.

[k]These women reported not having received any treatment or did not answer.

[l]Two women reported being treated with excisional treatment. The remaining women reported not having received any treatment.

[m]One woman reported being treated with excisional treatment. The remaining women reported not having received any treatment or did not answer.

hysterectomy in 19 cases and with chemotherapy/radiotherapy in one case. The remaining women were not treated or did not give an answer (Table 2). One woman, who reported being treated with excisional treatment in the past was found to have CIN3 during the GRECOSELF project. Of note, one woman with a negative Pap test before the previous three years was diagnosed with invasive SCC during the study (Table 3).

Among women who reported having a Pap test within 3 years prior to their recruitment in the GRECOSELF study ($N = 9{,}936$, 77.7%), abnormal results ($N = 421$, 3.3%) were reported to have led to 93 cases with excisional treatment and 41 hysterectomies (Table 2). Within the group of women with a reported positive Pap test within the previous three years, self-sampling led to the detection of seven cases with CIN2+ (three cases of CIN2 and four cases of CIN3), of which one woman reported having been treated with excisional treatment. During the GRECOSELF study, among women reporting a negative Pap test within the previous 3 years, there was one diagnosed with invasive cervical adenocarcinoma (Table 3).

HPV testing parameters in different age groups

Table 4 presents HPV positivity rates, positive predictive value (PPV), and CIN2+ detection rates, as well as compliance with colposcopy referral rates among women of different age groups. Overall, hrHPV testing positivity rate progressively decreased with increasing age. This rate was 20.7%, 10.6%, 6.7%, and 5.1% for women 25–29, 30–39, 40–49, and 50–60 years old respectively. A similar trend was observed regarding all three channels of the Cobas HPV test corresponding to positivity for HPV16, HPV18, and for the 12 other hrHPVs. The detection rates for CIN2+ also decreased with increasing age. Among women positive for any of the 14 hrHPVs this rate decreased from 1.4% in

Table 3. Cervical cancer screening history of the women with high-grade cervical disease, VaIN, or cervical cancer participating in the study

Screening History	CIN2 *N*(%[a])	CIN3 *N*(%[a])	VaIN *N*(%[a])	AIS *N*(%[a])	AC *N*(%[a])	SCC *N*(%[a])	Total *N*(%[a])
Women reporting NEVER having a Pap test	3 (10.7%)	2 (4.5%)	—	—	—	—	5 (6.5%)
Women reporting having a Pap test before the previous 3 years	2 (7.1%)	10 (22.7%)	—	—	—	1 (100.0%)	13 (16.9%)
Women reporting having a NEGATIVE Pap test before the previous 3 years	2 (7.1%)	9 (20.4%)	—	—	—	1 (100.0%)	12 (15.6%)
Women reporting having a POSITIVE[b] Pap test before the previous 3 years	—	1[c] (2.3%)	—	—	—	—	1 (1.3%)
Women reporting having a Pap test within the previous 3 years	23 (82.2%)	32 (72.8%)	2 (100.0%)	1 (100.0%)	1 (100.0%)	—	59 (76.6%)
Women reporting having a NEGATIVE Pap test within the previous 3 years	18 (64.3%)	27 (61.4%)	2 (100.0%)	1 (100.0%)	1 (100.0%)	—	49 (63.6%)
Women reporting having a POSITIVE[b] Pap test within the previous 3 years	3[d] (10.7%)	4[e] (0.9%)	—	—	—	—	7 (0.9%)
Total	28 (100.0%)	44 (100.0%)	2 (100.0%)	1 (100.0%)	1 (100.0%)	1 (100.0%)	77 (100.0%)

Abbreviations: AIS, adenocarcinoma *in situ*; AC, invasive adenocarcinoma; SCC, squamous cervical carcinoma.

[a]All percentages are calculated per column. Numbers in columns do not sum up due to missing values.

[b]Positive pap test was defined in the questionnaire as a result requiring referral to the gynecologist for further evaluation. Inflammation, metaplasia and other conditions apart from dysplastic cellular abnormalities were excluded.

[c]This woman reported that she was treated with excisional treatment.

[d]No woman reported receiving treatment.

[e]One woman reported being treated with excisional treatment, and 3 women reported not receiving treatment.

women aged 25–29 years to 0.2% in women over 50 years, and similar decrease was observed among women positive for HPV16 and/or 18 (regardless of positivity for the 12 other hrHPVs; 0.9% for the youngest women of the study cohort and 0.1% for the oldest).

Positive predictive value of hrHPV testing was lowest for women 50–60 years old (5.0%) and highest for women 40–49 (11.6%). Positive predictive value of HPV16/18 genotyping, however, was highest for the youngest women, aged between 25 and 29 (27.0%) and lowest for the oldest ones (11.8%).

Women, in general, accepted the fact that they were referred to have a colposcopy and about two out of three attended their colposcopy appointment. However, there was a drop-out rate from colposcopy referral which ranged from 31.4% for women aged between 25 and 29 years, to 23.7% for women between 40 and 49 years.

Discussion

The main objective of the GRECOSELF study was to explore the implementation of self-sampling combined with hrHPV DNA testing in Greece, utilizing a nationwide midwifery network to approach women residing in remote rural areas of the country who consequently do not have regular access to cervical cancer screening. Self-sampling is considered a paradigm shift in cervical cancer control (11); an idea which, although not new to the scientific community, was only recently incorporated in the big picture of cervical cancer prevention. However, there are still several issues which require attention and further research. Particularly, it has been suggested that there is a need to set up pilots before national roll out of self-sampling strategies due to the availability of different hrHPV assays and sampling devices combined with the scarcity of self-sampling studies on the general screening population and the variability of response rates among screening nonattenders and hard-to-reach populations (12). The GRECOSELF project serves this purpose in Greece, a country without an organized cervical cancer screening program to-date, where screening coverage is low and based on the cervical smear test (7).

In GRECOSELF we used self-sampling with a dry swab combined with hrHPV DNA testing using the Cobas HPV test. This combination serves, first, the use of a PCR-based HPV test, and, second, the use of a sampling process which is as easy as possible. Regarding PCR-based HPV tests, a meta-analysis has shown that such tests, validated already for primary cervical cancer screening on physician-collected samples, yield not statistically different sensitivity and specificity for the detection of CIN2+ when self-collected or physician-collected samples are used, regardless of the sampling device (brush or lavage) (6). This meta-analysis has been recently updated including novel tests, again documenting that hrHPV PCR-based assays are similarly accurate regardless of the sample collection method (self-sampling or physician-sampling; ref. 12). Concerning the sampling method, the dry swab is reliable, easy to use and of low-cost requiring women to follow very simple sampling and storage instructions (13, 14). In our

Table 4. HPV test positivity rate, compliance with colposcopy referral, PPV, and CIN2+ detection rate as per age group

Age Group	14hrHPV+[a], n/N(%)	12hrHPV+[b], n/N(%)	HPV16+[c], n/N(%)	HPV18+[d], n/N(%)	Compliance with colposcopy referral[e], n/N(%)	PPV (14hrHPV+)[f], n/N(%)	CIN2+ (14hrHPV+) DR[g], n/N(%)	PPV (HPV16/18+)[h], n/N(%)	CIN2+ (HPV16/18+) DR[i], n/N(%)
25–29	223/1,098 (20.3%)	194/1,098 (17.7%)	46/1,098 (4.2%)	14/1,098 (1.3%)	153/223 (68.6%)	15/153 (9.8%)	15/1,098 (1.4%)	10/37 (27.0%)	10/1,098 (0.9%)
30–39	360/3,455 (10.4%)	280/3,455 (8.1%)	93/3,455 (2.7%)	30/3,455 (0.9%)	251/360 (69.7%)	29/251 (11.5%)	29/3,455 (0.8%)	20/86 (23.3%)	20/3,455 (0.6%)
40–49	249/3,770 (6.6%)	206/3,770 (5.5%)	53/3,770 (1.4%)	16/3,770 (0.4%)	190/249 (76.3%)	22/190 (11.6%)	22/3,770 (0.6%)	10/53 (18.9%)	10/3,770 (0.3%)
50–60	238/4,788 (4.9%)	201/4,788 (4.2%)	32/4,788 (0.7%)	18/4,788 (0.4%)	179/238 (75.2%)	9/179 (5.0%)	9/4,788 (0.2%)	4/34 (11.8%)	4/4,788 (0.1%)
Overall	1070/12,844 (8.3%)	881/12,844 (6.8%)	224/12,844 (1.7%)	78/12,844 (0.6%)	773/1,070 (72.2%)	75/773 (9.7%)	75/12,844 (0.6%)	44/210 (20.9%)	44/12,844 (0.3%)

Abbreviations: CIN2+ DR, cervical intraepithelial neoplasia grade 2 or worse detection rate; hrHPV, high-risk human papillomavirus; PPV, positive predictive value.

[a]This variable refers to positivity for at least one of the 14 high-risk HPVs detected by the Cobas HPV test. Nominator is the number of 14hrHPV+ women and denominator the total number of women per age group.

[b]This variable refers to positivity for at least one of the 12 high-risk HPVs (non HPV16/18) detected by the Cobas HPV test, without excluding co-infections. Nominator is the number of 12hrHPV+ women and denominator the total number of women per age group.

[c]This variable refers to positivity for HPV16, without excluding coinfections. Nominator is the number of HPV16+ women and denominator the total number of women per age group.

[d]This variable refers to positivity for HPV18, without excluding coinfections. Nominator is the number of HPV18+ women and denominator the total number of women per age group.

[e]This variable refers to the percentage of 14hrHPV+ women who were subjected to colposcopy. Nominator is the number of 14hrHPV+ women who were subjected to colposcopy and denominator the total number of hrHPV+ women per age group.

[f]This variable refers to the PPV of 14hrHPV positivity. Nominator is the number of women with CIN2+ (VAIN cases not included) and denominator the total number of hrHPV+ women who were subjected to colposcopy per age group.

[g]This variable refers to the detection rate of CIN2+ (VaIN cases not included) according to 14hrHPV positivity. Nominator is the number of women with CIN2+ and denominator the total number of women per age group.

[h]This variable refers to the PPV of HPV16/18 positivity. Nominator is the number of women (positive for HPV16 and/or 18) with CIN2+ (VAIN cases not included) and denominator the total number of women (positive for HPV16 and/or 18) who were subjected to colposcopy per age group.

[i]This variable refers to the detection rate of CIN2+ (VaIN cases not included) according to HPV16/18 positivity. Nominator is the number of women with CIN2+, who were positive for HPV16 and/or 18, and denominator the total number of women per age group.

study, the dry swab was used without any restrictions regarding storage and transportation after an initial assessment phase with different temperature conditions in storing and transporting the samples, hence simplifying the sampling process so that barriers to implementation were minimized.

Overall, the GRECOSELF study yielded hrHPV positivity rates for each of the three channels of the Cobas HPV test similar to previous studies for the Greek female population (7, 10), which indicates that the combination of this test with the dry swab, stored and transported in ambient temperature according to the GRECOSELF protocol, is reliable in detecting hrHPV infection within the study population. Moreover, the decrease in the observed hrHPV positivity rates with increasing age, presented herein, is a fact in HPV epidemiology worldwide (15). A difference is that in our cohort, we did not observe the slight increase of hrHPV positivity rates in women older than 50 years, which is observed in most areas of the world (15) and also reported in a previous study in Greece (16). Similarly, CIN2+ detection rate among women who participated in GRECOSELF was 0.6%, a figure also in line to the ones reported in previous studies from Greece (10, 17). Of note, the detection of 75 women with prevalent high-grade cervical disease or cancer, informed by hrHPV testing, indicates that the current opportunistic cervical cancer screening for the rural Greek female population is inadequate and that the proposed strategy implemented in GRECOSELF has a positive impact.

An important parameter regarding self-sampling implementation is how to reach the target population. In GRECOSELF, we used a network of midwives, working in public health care, who cover most of the rural areas of the country, to serve as providers for self-sampling devices. The model was a combination of a community campaign at the village level, involving local authorities, and a door-to-door approach. Regional Health Administrations promoted this initiative by providing directions to local primary care physicians to facilitate the work conducted by midwives. The use of healthcare workers is a promising method to increase cervical cancer screening coverage; a recent meta-analysis compared different methods to increase coverage, and among them the door-to-door distribution of self-sampling kits by healthcare workers was the one that achieved the highest participation rates against the mailing of self-sampling kits directly to the home or expecting women to opt-in for self-sampling (12). However, the way that self-sampling is provided to screening nonattenders should be tailored according to the specific needs of and resources available to the target population. Regarding Greece and according to the results presented herein the midwifery network suggested by GRECOSELF is a promising means of increasing screening coverage, given the geography of the country as well as the

already established primary health care setting. Moreover, in agreement with the international literature (18, 19), we showed that the compliance to colposcopy referral rate after a HPV-positive self-collected sample is acceptable, because the majority of women referred to colposcopy actually had one.

The main strengths of the GRECOSELF study are, first, the large sample size (the largest and widest thus far in Greece reporting on cervical cancer screening with hrHPV testing and self-sampling); and, second, the active utilization of an already established network of midwives, who serve as public health care workers across the country. This was decided to facilitate the participation of women in self-sampling for cervical cancer screening, especially in remote areas with difficulties in access to health care, in a country without a nationally organized call–recall screening system. Limitations of the study are, first, the fact that, due to various reasons, not all hrHPV-positive women detected during the study accepted to undergo colposcopy; and, second, that cervical cancer screening history information was provided by the women themselves rather than documented reports, mainly due to the lack of registry infrastructure in Greece. Missing information regarding cervical cancer screening history was limited to very few participants, and screening coverage differed between geographic areas. Of note, all hrHPV-positive women were referred to colposcopy without triage. This is not what is suggested by GRECOSELF and it was decided only for research purposes as in previous studies (10, 20), to maximize CIN2+ detection rate in the framework of a cross-sectional study, given the fact that the study could not follow up on the status of these women.

In conclusion, the GRECOSELF study documented that, regarding cervical cancer secondary prevention for women residing in remote areas in Greece, the detection of hrHPV DNA with the Cobas HPV test on self-collected cervicovaginal samples using a dry cotton swab is a promising method as opposed to cytology-based opportunistic screening. The usage of a midwifery network for providing self-sampling kits could serve as an option for women, especially for countries where a national call–recall cervical cancer screening program seems as not a perspective for the near future.

Disclosure of Potential Conflicts of Interest

K. Chatzistamatiou has received speakers bureau honoraria from Roche Diagnostics Hellas. A.M Kaufmann has received speakers bureau honoraria from Roche, has unpaid consultant/advisory board relationship with GSK and MSD. No potential conflicts of interest were disclosed by the other authors.

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Acknowledgments

The study has been funded by Roche Diagnostics Hellas who has provided kits and consumables and covered sample transportation costs. The authors thank all the medical, paramedical, and technical personnel who voluntarily contributed to the realization of this study. We especially thank all the participating midwives, members of the Midwifery Societies of Thessaloniki, Thrace, Ioannina, Larisa, Nafplion, and Crete who voluntarily cooperated and managed to accomplish the difficult task of contacting women, informing them and collecting the samples and the questionnaires. We cordially thank the Directors of the Second, Third, Fourth, Fifth, Sixth, and Seventh Regional Health Administrations of Greece for their kind support. The authors acknowledge the contribution of the collaborators: Fani Fitsiou for technical support with cytology and Artemis Sevdali for administrative work. The authors especially thank Prof. C.J.L.M. Meijer (Department of Pathology, VU University Medical Center, Amsterdam, the Netherlands) for his generous assistance in the preparation of this manuscript. The authors also acknowledge the kind contribution of Mrs. Irini Lekka and Mr. Andrew Harisson regarding the editing of the manuscript.

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Received April 12, 2019; revised June 24, 2019; accepted August 8, 2019; published first August 19, 2019.

References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12–9.
2. Basu P, Ponti A, Anttila A, Ronco G, Senore C, Vale DB, et al. Status of implementation and organization of cancer screening in The European Union Member States-Summary results from the second European screening report. Int J Cancer 2018;142:44–56.
3. U.S. Preventive Services Task Force, Curry SJ, Krist AH, Owens DK, Barry MJ, Caughey AB, et al. Screening for cervical cancer: US Preventive Services Task Force Recommendation Statement. JAMA 2018;320:674–86.
4. Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, et al. Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine 2012;30Suppl 5:F88–99.
5. Ronco G, Dillner J, Elfstrom KM, Tunesi S, Snijders PJ, Arbyn M, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet 2014;383:524–32.
6. Arbyn M, Verdoodt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L, et al. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol 2014;15:172–83.
7. Agorastos T, Chatzistamatiou K, Zafrakas M, Siamanta V, Katsamagkas T, Constantinidis TC, et al. Epidemiology of HPV infection and current status of cervical cancer prevention in Greece: final results of the LYSISTRATA cross-sectional study. Eur J Cancer Prev 2014;23:425–31.
8. Rao A, Young S, Erlich H, Boyle S, Krevolin M, Sun R, et al. Development and characterization of the cobas human papillomavirus test. J Clin Microbiol 2013;51:1478–84.
9. Koliopoulos G, Nyaga VN, Santesso N, Bryant A, Martin-Hirsch PP, Mustafa RA, et al. Cytology versus HPV testing for cervical cancer screening in the general population. Cochrane Database Syst Rev 2017;8:CD008587.
10. Agorastos T, Chatzistamatiou K, Katsamagkas T, Koliopoulos G, Daponte A, Constantinidis T, et al. Primary screening for cervical cancer based on high-risk human papillomavirus (HPV) detection and HPV 16 and HPV 18 genotyping, in comparison to cytology. PLoS One 2015;10:e0119755.
11. Franco EL. Self-sampling for cervical cancer screening: Empowering women to lead a paradigm change in cancer control. Curr Oncol 2018;25:e1–e3.
12. Arbyn M, Smith SB, Temin S, Sultana F, Castle P, Collaboration on Self Sampling and HPV Testing, , et al. Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses. BMJ 2018;363:k4823.
13. Eperon I, Vassilakos P, Navarria I, Menoud PA, Gauthier A, Pache JC, et al. Randomized comparison of vaginal self-sampling by standard vs. dry swabs for human papillomavirus testing. BMC Cancer 2013;13:353.
14. Jun JK, Lim MC, Hwang SH, Shin HY, Hwang NR, Kim YJ, et al. Comparison of DRY and WET vaginal swabs with cervical specimens in Roche Cobas 4800 HPV and Abbott RealTime High Risk HPV tests. J Clin Virol 2016;79:80–4.
15. de Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007;7:453–9.
16. Agorastos T, Lambropoulos AF, Sotiriadis A, Mikos T, Togaridou E, Emmanouilides CJ. Prevalence and distribution of high-risk human papillomavirus in Greece. Eur J Cancer Prev 2009;18:504–9.
17. Chatzistamatiou K, Moysiadis T, Angelis E, Kaufmann A, Skenderi A, Jansen-Duerr P, et al. Diagnostic accuracy of high-risk HPV DNA genotyping for primary cervical cancer screening and triage of HPV-positive women, compared to cytology: preliminary results of the PIPAVIR study. Arch Gynecol Obstet 2017;295:1247–57.
18. Gupta S, Palmer C, Bik EM, Cardenas JP, Nunez H, Kraal L, et al. Self-sampling for human papillomavirus testing: increased cervical cancer screening participation and incorporation in international screening programs. Front Public Health 2018;6:77.
19. Sultana F, English DR, Simpson JA, Drennan KT, Mullins R, Brotherton JM, et al. Home-based HPV self-sampling improves participation by never-screened and under-screened women: Results from a large randomized trial (iPap) in Australia. Int J Cancer 2016;139:281–90.
20. Castle PE, Stoler MH, Wright TC Jr, Sharma A, Wright TL, Behrens CM. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. Lancet Oncol 2011;12:880–90.