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Reflex cytology for triage of high-risk human papillomavirus positive self-sampled material in cervical cancer screening: a prospective cohort study

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Objective High-risk human papillomavirus (HrHPV)-positive women detected by self-sampling require an extra visit at the general practitioner for additional cytology testing, but the loss to follow up within this triage is substantial. The aim of this study was to evaluate the clinical utility of reflex cytology on hrHPV-positive self-samples for immediate stratification of women who need referral for colposcopy.

Design A prospective cohort study.

Setting Two Dutch cervical cancer-screening laboratories.

Population 1014 screenees who tested hrHPV-positive on self-samples between 1 December 2018 and 1 August 2019.

Methods Self-samples were directly used for cytological analysis. Cytological and histological outcomes during follow up were obtained from the Dutch Pathology Registry (PALGA).

Main outcome measures Test performance of reflex cytology on self-samples was determined for different thresholds and compared with physician-taken cytology and histological outcomes.

Results Reflex cytology on self-samples for detecting abnormal cytology showed a sensitivity of 26.4% (95% CI 21.8–31.3) and specificity of 90.5% (95% CI 87.7–92.8). Of all ≥CIN2 cases, 29.4% (95% CI 22.5–37.1) were detected with reflex cytology on self-samples. The positive predictive value for detection of ≥CIN2 was higher with cytology on self-collected samples than on physician-collected samples. Of women who were lost to follow up, 12.9% were found to have abnormal cytology on their self-sampled material.

Conclusion Cytology testing is achievable on hrHPV-positive self-samples, could decrease the loss to follow up in screening and is easily implementable in the current clinical practice. Of all hrHPV-positive women with abnormal cytology on additional physician-collected samples, 26.4% could have been directly referred for colposcopy if triage with reflex cytology on self-sampled material had been performed.

Keywords Cervical cancer, cervical intraepithelial neoplasia, human papillomavirus, screening, self-sampling, triage.

Tweetable abstract Reflex cytology for triage of hrHPV$^+$ self-samples is of added value for direct referral of women for colposcopy.

Linked article This article is commented on by KJ Denton, p. 1663 in this issue. To view this mini commentary visit https://doi.org/10.1111/1471-0528.16388

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Introduction

In 2017, the Netherlands replaced cytomorphologically based cervical cancer screening by high-risk human papillomavirus (hrHPV) screening. hrHPV-negative women are retested in 5–10 years and hrHPV-positive women are additionally tested with cytology. Women with abnormal cytology are referred for colposcopy, whereas women with normal cytology are retested at 6 months (Figure 1). More than 50% of women diagnosed with cervical cancer have not been screened before and it therefore is of paramount importance to increase participation.[1,2] A meta-analysis has shown that non-responders may take part in screening if self-sampling is offered.[3] A large randomised trial has shown comparable accuracy between self-collected and physician-collected samples in detection of ≥CIN2 (cervical intraepithelial neoplasia) using polymerase chain reaction-based hrHPV-assays.[4] Additionally, self-samples are user-friendly alternatives for regular smears and are highly acceptable to women.[5,6] Therefore, in the hrHPV-based screening programme, all women may request a self-sampling device (Evalyn® Brush; Rovers® Medical Devices B.V., Oss, the Netherlands); ~7% of the participated women used a self-sample in 2017–2018.[7,8] It is expected that more women will perform self-sampling instead of getting a physician-sample in coming screening years.

Women who test hrHPV-positive on their self-sample are required to visit their general practitioner for additional cytology testing (Figure 1). It is believed that cytology is not achievable on self-samples, as it only contains a mixture of vaginal and exfoliated cervical cells. However, the loss to follow up among women preferring self-sampling is substantial. Only 78% of the hrHPV-positive self-sampled women were compliant to cytology triage within the following 15 months.[8] Therefore, screening via self-sampling could be further optimised if additional cytology testing was not needed. This is especially the case because women who screen hrHPV-positive on their self-sample have a higher risk of having ≥CIN2.[8]

Only a limited number of studies have compared the accuracy of cytological assessment between self-sampled and physician-sampled material, and have tested different types of self-sample devices, such as swabs, brushes and lavages.[9–14] They generally show a fair to moderate agreement and lower sensitivity on self-sampled material. However, reflex cytology on self-samples could be used as an additional step to identify women directly who need a colposcopy referral. This could improve patient satisfaction, reduce diagnostic delay and loss to follow up.

The aim of this study was to evaluate the clinical utility of cytology testing on hrHPV-positive self-samples for immediate stratification of women who need referral for colposcopy. We compared the performance of reflex cytology on self-samples with the cytology results of physician-samples and their histological outcomes.

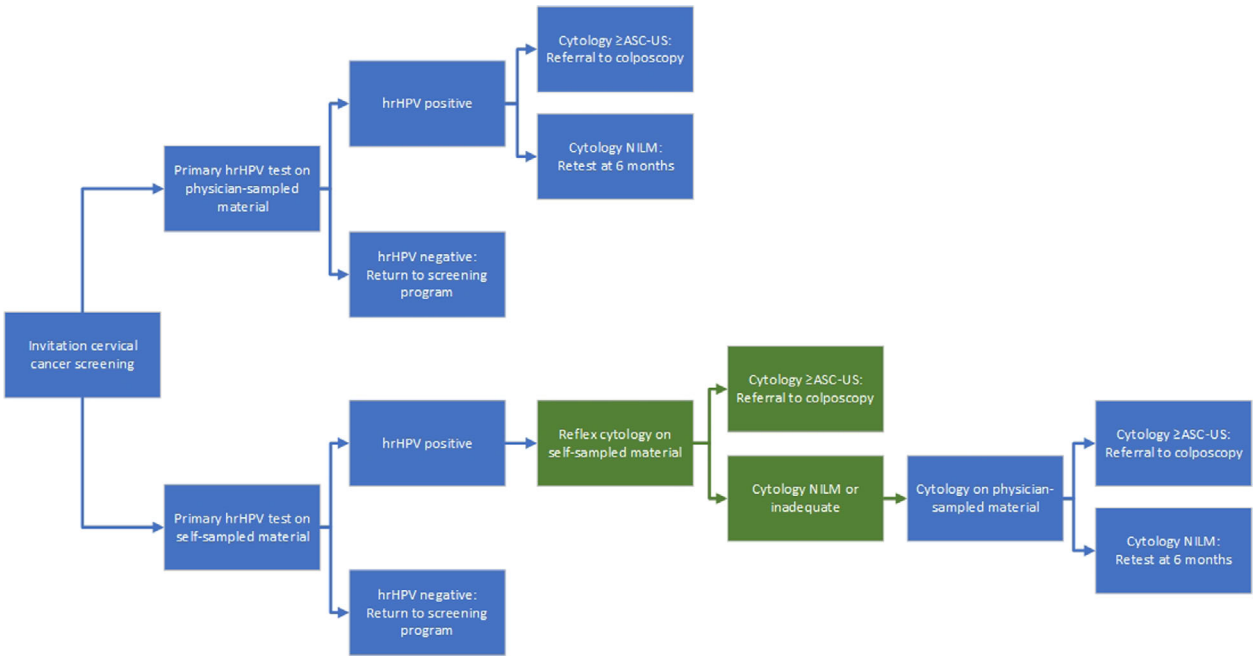

Figure 1. Flow-chart of possible screening algorithm with additional triage of hrHPV-positive women on self-sampled material (green boxes). ASC-US, atypical squamous cells of undetermined significance; hrHPV, high-risk human papillomavirus; NILM, negative for intraepithelial neoplasia or malignancy.

Methods

hrHPV-positive women who attended the cervical cancer screening programme by self-sampling with the Evalyn® Brush between December 2018 and August 2019 were included in this prospective cohort study (see Appendix S1 for the leaflet 'Instructions for using the self-sampling device' given to these women). We collected self-samples from Radboud university medical center, Nijmegen, and Pathology-DNA, Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands, both institutes appointed as screening facilities. By law, samples were stored at room temperature for 3 months for possible additional testing during the regular management of these women and for quality purposes. Women participating in the screening programme were informed that residual material could be used for anonymous research and they had the opportunity to opt out. The regional institutional review board and the National Institute for Public Health and the Environment granted approval before start of the study (No. 2018-4901). The self-samples of all hrHPV-positive women who did not opt out were recoded and analysed anonymously. Patients or the public were not involved in the development of the research.

As part of the regular screening, women who tested hrHPV-positive on their self-sample were invited to have an additional smear taken by their general practitioner for triage cytology testing. Women with ≥ASC-US were referred for colposcopy, whereas women with normal cytology were invited for retesting at 6 months. The colposcopy was performed according to the Dutch national guidelines and the decision for diagnostic biopsy or treatment was the responsibility of the individual colposcopist. The self-samples were processed in the same way as the physician-samples are processed for cytology. The residual material of the hrHPV-positive self-samples was stored in preservation medium as per the manufacturer's instructions (Preservcyt Transport Medium; Cytyc Corporation, Boxborough, MA, USA). Microscopic slides were prepared using the ThinPrep 5000 Processor (Hologic Inc, Marlborough, MA, USA) and stained with Papanicolaou stain. Seven trained cytotechnologists reviewed the ThinPrep self-sampling slides and were unaware of clinical data, such as cytological and histological outcomes. The regular ThinPrep slides were assessed as usual by a cytotechnician for diagnostic healthcare. The cytological and histological outcomes during follow up were obtained from the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA; Houten, the Netherlands) until December 2019. The cytological classification was performed according to the Dutch CISOE-A classification. For analysis, the CISOE-A classification system was translated into the Bethesda nomenclature; in which Pap0 equals inadequate cytology, Pap1 normal cytology, Pap2 atypical squamous cells of undetermined significance (ASC-US), Pap3a1 low-grade squamous intraepithelial lesion (LSIL), and Pap3a2, Pap3b or Pap4 high-grade squamous intraepithelial lesion (HSIL).[15] The histological specimens were classified according to the CIN histological grading system. A relevant core outcome set is not available yet in the 'Core Outcomes in Women's and Newborn Health' (CROWN) database and therefore not used in this study.

The sensitivity, specificity and predictive values of cytology testing on self-samples were determined for thresholds of low-grade (≥ASC-US) and high-grade cytology (HSIL) compared with cytology results on subsequent physician-collected samples and histological outcomes during follow up.

Descriptive statistics were used to calculate the sensitivity, specificity and predictive values with corresponding 95% confidence intervals. Exact Clopper-Pearson confidence intervals were used for sensitivity and specificity and the standard logit confidence intervals for the predictive values. The overall agreement of self-sampled and physician-sampled material was statistically tested using percentage agreements and Cohen's Kappa statistics. Analyses were carried out using SPSS statistical software version 22.0 (IBM SPSS Statistics, New York, NY, USA).

Funding

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Results

Of all self-samples, 7.7% tested hrHPV-positive and these 1014 cases were all included in this study, without selection. Of the 1014 women, 82 women (8.1%) had no subsequent cytology smear taken and thus were lost to follow up. The remaining 932 women (91.9%) had an additional cytology test taken by their general practitioner, of whom 564 women had normal cytology, 221 women had a low-grade cytology result, 144 women a high-grade cytology result and three women had an inadequate Pap test result (Table 1). The median time between self-sampling and additional cytology was 22 days (range 3–312), and between referral advice and histology 28 days (range 0–288). Of the 365 women with a referral advice (≥ASC-US; 39.2%) for colposcopy, there was no biopsy taken in 95 women (26.0%), 110 women (30.1%) had ≤CIN1 and 160 women (43.8%) had ≥CIN2 on histology (Figure 2). The median time between self-sampling and histology was 57 days (range 17–308).

The cytological results from self-sampling are plotted against the cytologic results from the first physician-collected samples during follow up in Table 1. Most cytology from self-sampled material was sufficient for analysis,

Table 1. Cytological results of hrHPV-positive self-collected samples versus first physician-collected samples during follow up

Self-collected sample	First physician-collected sample during follow up					
	Normal *n* (%)	Low-grade* *n* (%)	High-grade** *n* (%)	Inadequate *n* (%)	Not done *n* (%)	Total *n* (%)
Normal	485 (86.0)	159 (71.9)	101 (70.1)	2 (66.7)	70 (85.4)	817 (80.6)
Low-grade*	41 (7.3)	48 (21.7)	19 (13.2)	1 (33.3)	9 (11.0)	118 (11.6)
High-grade**	10 (1.8)	5 (2.3)	21 (14.6)	0 (0)	1 (1.2)	37 (3.6)
Inadequate	28 (5.0)	9 (4.1)	3 (2.1)	0 (0)	2 (2.4)	42 (4.1)
Total	564 (55.6)	221 (21.8)	144 (14.2)	3 (0.3)	82 (8.1)	1014

ASC-H, atypical squamous cells cannot rule out high-grade squamous intraepithelial lesion; ASC-US, atypical squamous cells of undetermined significance; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.
*Low-grade cytology: ASC-US or LSIL.
**High-grade cytology: ASC-H or HSIL.

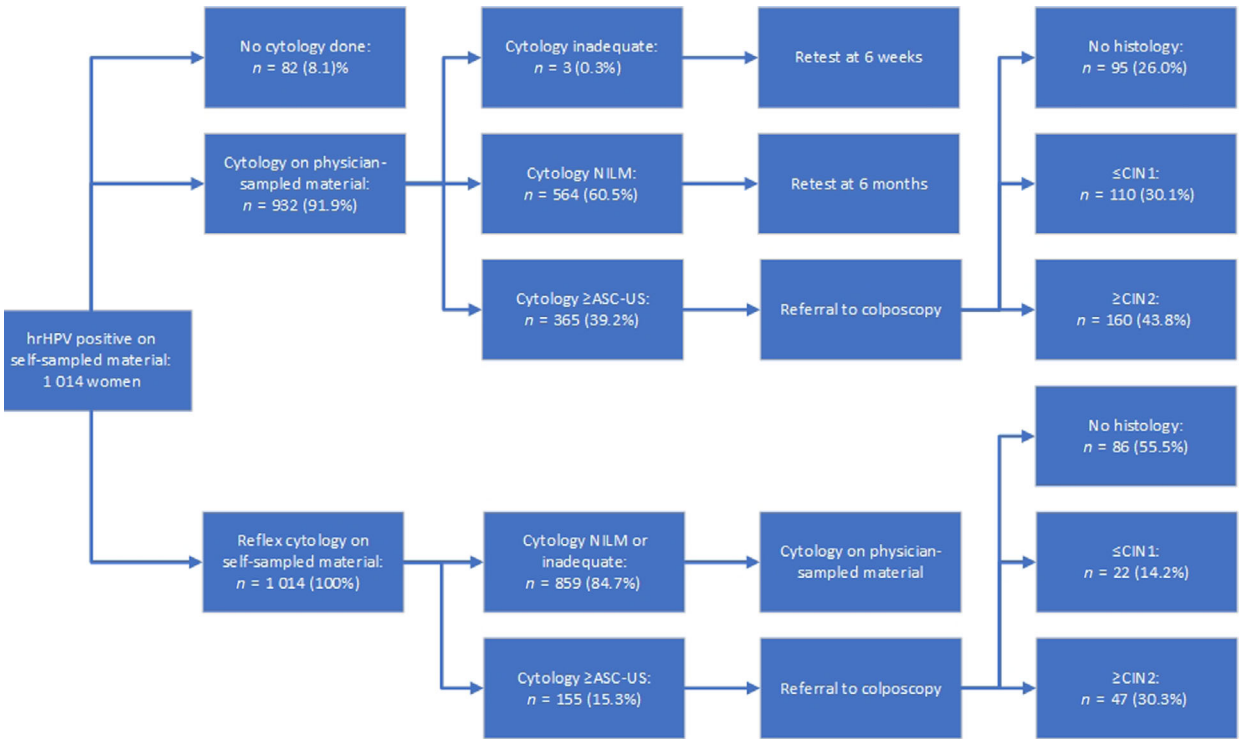

Figure 2. Study flow-chart. ASC-US, atypical squamous cells of undetermined significance; hrHPV, high-risk human papillomavirus; NILM, negative for intraepithelial neoplasia or malignancy.

with only 4.2% of the self-samples being of unsatisfactory quality, due to low cellularity (<4 cells per field at 40× magnification or <5000 cells per slide). Of the self-collected samples with satisfactory quality for cytology testing, 155 samples (15.9%) were identified as showing abnormal cytology (Table 1). The overall agreement between normal and abnormal cytology for self-sampled versus physician-sampled material was 65.0% (κ = 0.19, 95% CI 0.13–0.24). The clinical utility of using reflex cytology on hrHPV-positive self-sampled material as triage for detecting ≥ASC-US or HSIL is shown in Table 2. Of all hrHPV-positive women with ≥ASC-US on subsequent physician-sampling, 26.4% could be referred directly for colposcopy by triage with reflex cytology on self-sampling. The specificity was high

Table 2. Sensitivity, specificity and predictive values of cytology on self-collected samples in the triage of hrHPV-positive women for detection of ≥ASC-US, HSIL, ≥CIN2 and ≥CIN3

Test	TP *n*	FP *n*	TN *n*	FN *n*	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Self-collected sample versus first physician-collected sample during follow up								
For detection of ≥ASC-US	93	51	485	260	26.4 (21.8–31.3)	90.5 (87.7–92.8)	64.6 (57.1–71.4)	65.1 (63.5–66.6)
For detection of HSIL	21	15	733	120	14.9 (9.5–21.9)	98.0 (96.7–98.9)	58.3 (42.5–72.6)	85.9 (85.1–86.8)
Self-collected sample versus highest physician-collected sample during follow up								
For detection of ≥ASC-US	99	45	453	292	25.3 (21.1–29.9)	91.0 (88.1–93.3)	68.8 (61.4–75.3)	60.8 (59.3–62.3)
For detection of HSIL	21	15	726	127	14.2 (9.0–20.9)	98.0 (96.7–98.9)	58.3 (42.5–72.6)	85.1 (84.3–85.9)
Self-collected sample versus highest histological value during follow up								
Threshold ≥ASC-US								
For detection of ≥CIN2	47	22	94	113	29.4 (22.5–37.1)	81.0 (72.7–87.7)	68.1 (57.8–77.0)	45.4 (42.1–48.7)
For detection of ≥CIN3	32	37	130	77	29.4 (21.0–38.9)	77.8 (70.8–83.9)	46.4 (36.5–56.5)	62.8 (59.3–66.1)
Threshold HSIL								
For detection of ≥CIN2	21	2	114	139	13.1 (8.3–19.4)	98.3 (93.9–99.8)	91.3 (71.5–97.8)	45.1 (43.5–46.7)
For detection of ≥CIN3	17	6	161	92	15.6 (9.4–23.8)	96.4 (92.3–98.7)	73.9 (53.6–87.4)	63.6 (61.6–65.6)

≥ASC-US, atypical squamous cells of undetermined significance or worse; CI, confidence interval; ≥CIN2, cervical intraepithelial neoplasia grade 2 or worse; ≥CIN3, cervical intraepithelial neoplasia grade 3 or worse; FN, false negatives; FP, false positives; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; NPV, negative predictive value; PPV, positive predictive value; TN, true negatives; TP, true positives.

for detecting ≥ASC-US or HSIL (90.5 and 98.0%, respectively). The clinical utility of using reflex cytology on hrHPV-positive self-sampled material for detecting ≥CIN2 or ≥CIN3 is shown in Table 2 as well. For both ≥CIN2 and ≥CIN3 cases, 29.4% were detected directly with reflex cytology on self-samples. The positive predictive value (PPV) for detection of ≥CIN2 and ≥CIN3 was higher with cytology on self-collected samples than on physician-collected samples for both thresholds of ≥ASC-US and HSIL: PPV of detecting ≥CIN2 with threshold ≥ASC-US 68.1% versus 59.3%, respectively; PPV of detecting ≥CIN2 with threshold HSIL 91.3% versus 86.8%, respectively; PPV of detecting ≥CIN3 with threshold ≥ASC-US 46.4% versus 39.6%, respectively; PPV of detecting ≥CIN3 with threshold HSIL 73.9% versus 64.7%, respectively.

Fifty-one cases (9.5%) were scored as ≥ASC-US on self-sampling but had normal cytology on the first physician-sample during follow up. With retesting at 6 months, six cases showed ≥ASC-US, 12 women still had normal cytology, and 33 women lost to follow up. At the same time, we found 11 cases (12.9%) of ≥ASC-US on self-sampling for which no physician-collected sample was taken or where it was inadequate for analysis (lost to follow up).

Discussion

Main findings

In this study, we showed that reflex cytology on hrHPV-positive self-samples is achievable to detect cervical abnormalities in hrHPV-positive women attending screening. Importantly, >25% of the women with ≥ASC-US in their physician-taken smear and ~30% of the women with histological samples ≥CIN2 could have been referred directly for colposcopy without an additional physician-taken smear. Thus, reflex cytology on self-samples can be used as a direct triage test to identify women who could benefit from immediate referral, especially due to the high specificity. Also, not all false-positive women on self-sampling may be truly false-positive, as regular cytology has only a sensitivity of ~65% (range 30–87%).[16,17] For example, from the women with ≥ASC-US on self-sampling, but normal cytology on physician-sampling, one-third of the women with follow up showed cytological abnormalities at 6 months' follow up. In addition, the PPV for detecting ≥CIN2 and ≥CIN3 was higher for women with abnormal cytology on self-sampling than on physician-sampling. As it is expected that ≥CIN2 would exfoliate more abnormal cervical cells than ≤CIN1, once abnormal cervical cells are found in a self-sample, the chance of finding high-grade disease is higher. Furthermore, the lack of endocervical, endometrial and inflammatory cells makes the interpretation of the slide easier.

Strengths and limitations

Strengths of our study are its prospective design with a large and representative cohort of women who participate in the current Dutch screening programme through self-sampling. For example, in our study cohort, 60.5% of hrHPV-positive self-samples showed normal cytology in the physician-taken smear (Figure 2), compared with 62.4% in the Dutch screening programme in 2017–2018.[7,8] The

majority of women who participated in screening through self-sampling were women aged 30 years participating for the first time or previously participating women. Only 15% were former non-responders. A limitation of our study is that we do not have complete cytologic follow up at 6 months for the whole group of hrHPV-positive women with normal cytology on the physician-collected sample, as our follow up was between 3 and 13 months. We also only have histological follow up for a part of the referred study population, as women with low-grade cytological and colposcopic findings may be treated conservatively with cytological follow up only. In addition, as all self-samples were analysed anonymously, we did not have any information about the women. Features such as age could have been useful, as adequate cytology sampling is more difficult in older women.

A relevant core outcome set is not available yet in the Core Outcomes in Women's and Newborn Health (CROWN) database and therefore not used in this study. However, the Bethesda nomenclature and the CIN histologic grading system are internationally accepted terminologies.

Interpretation

Although reflex cytology on self-samples could be used as an additional triage test, it cannot replace additional cytology testing at the general practitioner after a hrHPV-positive self-sample. Women with abnormal cytology could be referred directly, but women with inadequate and normal cytology still need an additional cytology test at the general practitioner, as the negative predictive value is only 65.1%. Also, our overall agreement between normal and abnormal cytology between self-sampled material collected with the Evalyn® Brush and physician-sampled material collected with the Cervex-Brush® was poor (overall agreement 65%; $\kappa = 0.19$). The concordance between cytology testing on self-sampled and physician-sampled material has been studied in some small studies with a variety of self-sampling devices. The only other study that compared the Evalyn® Brush self-sampling device with the Cervex-Brush® sampled by the physician showed a good agreement between cytological results (overall agreement 76.8%; $\kappa = 0.57$),[13] although this study had less abnormalities in its study cohort. Other studies compared the Mermaid lavage method, the Kato lavage method and self-sampling with the Cytobroom with physician-sampled brushes with all different concordances.[10,11,14]

There are studies showing better accuracy for reflex cytology on self-collection compared with our study. A cross-sectional study found a sensitivity of 55.0% and specificity of 84.1% in self-sampled material collected with an endocervical brush compared with physician-sampled material for detecting ≥CIN2.[9] A case-control study between the Fournier self-sampling swab and physician-sampling showed comparable performance results for detecting ≥CIN1 (sensitivity 50–60.0% versus 65.3%; specificity 73.8–81.8% versus 81.0%, respectively).[12] However, as these self-sampling devices collect from different areas (high-vaginal or cervicovaginal) and in different ways, the results of our study cannot be extrapolated.

Sampling-error and screening-error could explain the high false-negative results. Self-samples are taken from the cervicovaginal area, not the squamocolumnar junction of the cervix. They contain a mixture of vaginal and exfoliated cervical cells, and usually do not show endocervical cells, although, the presence of endocervical cells is not a criterion for adequacy according to the guidelines. The possibility of detecting abnormalities is decreased when fewer abnormal cervical cells are present compared with the regular cervical smear. On the other hand, the advantage of analysing cervicovaginal samples is the lack of endocervical, endometrial and inflammatory cells, which makes the interpretation of the slide easier.

While cytology remains liable to subjective interpretation and morphological alterations of exfoliated cervical cells, molecular triage methods, such as HPV genotyping, methylation markers and microRNA detection, are more objective and reproducible.[18–20] Therefore, they are attractive alternative triage methods and may play an important future role in screening. However, more research is warranted on molecular testing on self-samples before it can be implemented in screening. Until then, reflex cytology on self-samples as an additional triage test could be an attractive alternative in the current screening programme to improve cervical cancer prevention further.

Only a few self-samples in our study were scored inadequate based on low cellularity ($n = 42$; 4.2%). Compared with 24 women (2.4%) initially with an inadequate Pap test result and 82 women (8.1%) without a cytology result from the physician, it is a great advantage to use reflex cytology on self-samples to reduce the loss to follow up during additional testing at the general practitioner. For example, 11 women (12.9%) with ≥ASC-US on self-sampling had an inadequate or absent physician-collected cytology result and could have also benefitted from this direct referral. A more definitive abnormal result (hrHPV-positive and ≥ASC-US) and direct referral to colposcopy may elicit better compliance to follow up. However, it is still not guaranteed that these women will show up for colposcopy. We found a different attendance rate between our study (91.9%) and the national as a whole (78%). This could be explained by demographic differences in compliance, as we only included two of five laboratories, or decreases in the loss to follow up over time, as self-sampling was only introduced in screening in 2017.

Another advantage of reflex cytology on self-sampled material is that it could be easily implemented in the

current clinical practice with low extra costs. Around 15% of the women could omit a visit to the general practitioner; this would reduce the loss to follow up and shorten delay in diagnostic work-up. It could also be an alternative for cervical screening in low-resource settings with a high risk of loss to follow up but sufficient cytology quality or in patients reluctant to undergo pelvic examination.

Conclusion

Cytology testing is direct applicable on hrHPV-positive self-samples and is of added value as a direct triage test for immediate referral stratification, which will improve patient satisfaction, reduce diagnostic delay and loss to follow up, and could be easily implemented in current clinical practice.

Disclosure of interests

None declared. Completed disclosure of interests forms are available to view online as supporting information.

Contribution to authorship

All the authors have made substantial contributions to the conception, design of the work, the acquisition, analysis or interpretation of data for the work. They have participated in drafting the manuscript and approval of the version to be published. Conception and design: DLL, JEMV, LFAGM, RLMB, AGS. Acquisition of data: DLL, JEMV, AJCB, AGS. Analysis of the samples: DLL, JEMV. Statistical analysis: DLL. Data interpretation: DLL, WJGM, JEMV, RLMB, AGS. Manuscript writing: DLL. Final approval of manuscript: DLL, WJGM, JEMV, AJCB, LFAGM, RLMB, AGS. Accountable for all aspects of the work: DLL, WM, JEMV, AJCB, LFAGM, RLMB, AGS.

Details of ethics approval

All the authors report adherence to ethical standards in the conception of the work, data collection and writing of the manuscript.

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IRB status

The study was approved by the regional institutional review board (the Research Ethics Committee of the Radboud University Medical Center Nijmegen) in October 2018 (No. 2018-4901).

Submission declaration and verification

This manuscript has not been published previously, is not under consideration for publication elsewhere and this publication is approved by all authors and the responsible authorities where the work has been carried out. This manuscript will not be published elsewhere in the same form.

Transparency statement

This manuscript is an honest, accurate and transparent account of the study being reported. No important aspects of the study have been omitted. Any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data sharing statement

Data used in this study are available from the corresponding author on reasonable request.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. The leaflet *Instructions for using the self-sampling device* used by the Dutch cervical cancer screening programme for women who request a self-sample device. ■

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