CHAPTER 10

General discussion and future perspectives
Limitations of the current cervical screening programme

Cytology-based cervical screening programmes have reduced the incidence of and mortality from cervical cancer. However, the decrease of cervical cancer incidence has leveled off. Approximately, cervical cancers are missed by the current screening programme and this could be the result of several limitations of cytology-based screening programmes such as low reproducibility of cytology and limited sensitivity of a single smear. This necessitates repeat cytology in case of borderline positive results and repeated screening rounds during a lifetime. Moreover, the suboptimal participation rate of 65% is reported as an important drawback of the present screening programme. In addition, no decreasing trend was observed in incidence of adenocarcinomas, while only the incidence of squamous cell carcinomas has decreased. This suggests that cytology-based screening may not prevent adenocarcinomas since its precursor lesions are not as well-defined as precursors of squamous cell carcinomas.

HPV testing as primary screening test

An important limitation of the regular cytology based screening programme is the suboptimal sensitivity (50-70%) of a single smear to detect cervical intraepithelial neoplasia grade 2 or worse (CIN2+). Therefore, much effort has been made to improve screening by implementing HPV testing as primary screening tool, as is reviewed in this thesis (Chapter 9). Based on the studies described in the review, the Ministry of Health of the Netherlands has decided to replace cytology by HPV testing as primary screening test in the Netherlands in 2016. This implementation comprises primary HPV screening with cytology triage in women who test HPV positive. Since a negative HPV test result gives a better protection against cervical cancer than a negative cytology result, screening intervals in the Netherlands are extended from the age of 40. Within the new screening programme, women are screened in five rounds (instead of the current seven rounds) at ages of 30, 35, 40, 50 and 60. Women who test HPV positive, but cytology negative at the age of 40, 50 or 60, are retested after five years because their CIN2+ risk is too high to delay re-screening. Cost-effectiveness calculations have shown that implementation of HPV testing as primary screening tool can improve cervical screening efficacy.

Conclusions:
The HPV test is a more objective and more sensitive screening test and protects better against cervical cancer and high-grade precursor lesions than cytology.

In the Netherlands, the Ministry of Health has decided that cytology will be replaced by HPV testing as primary screening tool in 2016.
Improving the screening coverage by HPV self-sampling

Another main limitation of the current screening programme is non-attendance. Thirty-five percent of all invitees do not respond to the screening invitation or the reminder sent thereafter. It is important to reach these non-attendees, as more than half of all invasive cervical cancers are found in women who had not adequately been screened.\textsuperscript{4,9,17} Several studies have shown that compliance to screening may be improved when offering HPV self-sampling to non-attendees.\textsuperscript{18-25} Our review (Chapter 2) showed that HPV self-sampling can be used as alternative for screening on clinician-obtained cervical samples in women who are reluctant to participate in screening programmes. This review demonstrated that HPV self-sampling is at least as sensitive for CIN2+ as cytology on a physician-taken smear, but often less specific.\textsuperscript{26} Moreover, some studies have shown that it can be as sensitive as HPV testing on a physician-collected cervical scrape.\textsuperscript{26} A more recent meta-analysis has shown that the relative sensitivity for CIN2+ of HPV self-sampling was 0.99 (95%CI 0.94-1.06) compared with cytology (threshold ≥BMD) and 0.89 (0.83-0.96) compared with physician-taken HPV testing.\textsuperscript{27} However, when PCR-based HPV tests were used, relative sensitivity and specificity of HPV self-sampling and HPV testing by a physician-taken smear were (almost) similar. Therefore, a validated combination of self-sampling device and HPV test should be used to ensure similar accuracy as obtained by HPV testing on physician-taken samples with regard to CIN2+/CIN3+.\textsuperscript{26,27} Moreover, it is important to study the performance of newly developed self-sampling devices with regard to participation rate and accuracy, as was demonstrated in this thesis (Chapter 3 and Chapter 4).

Differences in participation rates with HPV self-sampling may reflect differences between populations. For example differences on cultural, religious or education level could play a role.\textsuperscript{24,25} Indeed, a pooled analysis of two self-sampling studies in the Netherlands\textsuperscript{18,19}, in which non-attendees of screening were offered self-sampling, showed that response rates to self-sampling were especially influenced by ethnicity and screening history. Native Dutch women revealed a higher response rate than immigrant non-attendees. In all ethnic groups, never screened women had a higher response than under-screened women (last smear taken > 7 years before).

To increase screening coverage it is necessary to obtain information about women’s reasons for non-attendance to regular screening and whether they will participate when self-sampling is offered. The main reason for non-attendance described in this thesis (Chapter 5) was that invitees forgot to schedule an appointment at the physician for a cervical smear. In addition, women indicated that they felt too embarrassed or afraid to have a cervical smear taken by the physician. In other studies, practical barriers, such as difficulty to make an appointment and to fit this in their daily activities, were also found as reasons for non-attendance.\textsuperscript{28} Other reasons were emotional barriers, including a previous negative experience, embarrassment, and feelings of insecurity and anxiety.\textsuperscript{28,29} The mentioned advantages for women to participate when self-sampling devices are offered were privacy, ease, time and place of sampling.\textsuperscript{30-34} Our study among self-sampling participants (Chapter 5) reported convenience and self-control as most important reasons for attendance with HPV self-sampling.
In low- and middle-resource countries lacking medical services, HPV self-sampling has shown to facilitate access to cervical screening.\textsuperscript{35-37} A clinically validated and easy to perform HPV test should be used in these women, who are probably only screened once or a few times per lifetime.\textsuperscript{38,39}

**Conclusions:**

HPV self-sampling is at least as sensitive for CIN2+ as cytology on a physician-taken sample, but often less specific.

To reach similar accuracy of HPV testing on self- and physician-sampled material, a validated combination of self-sampling device and HPV test should be used.

Attendance rates will increase by offering HPV self-sampling to non-responders.

Paying more attention to women who forgot to visit the physician for a cervical scrape may also increase attendance rates.

Self-sampling could facilitate access to screening in low- and middle-resource countries.

**Molecular methylation markers as triage test in HPV positive women**

When replacing cytology by primary HPV based screening, a validated HPV test should be used that has proven to detect HPV infections associated with high-grade lesions.\textsuperscript{40} Even with a validated HPV test, the specificity of HPV testing is still 3-4\% lower than that of cytology.\textsuperscript{41} This is due to the inability of HPV testing to distinguish between transient infections and persistent infections that can progress to (high-grade precursor lesions of) cervical cancer. Therefore, triage tests are needed to identify those HPV positive women who have (pre)cancer and thus are in need of colposcopy (and treatment). Presently, cytology is mostly used as triage test on cervical smears of HPV positive women, with or without HPV16/18 genotyping.\textsuperscript{42-44}

Previously, it has been shown that some tumour suppressor genes involved in cervical cancer are inactivated by hypermethylation of their promoter region. Methylation marker panels, as described in this thesis (Chapter 6-8), are attractive candidates for triage of HPV positive women. Such markers have similar sensitivity as cytology, but the lesions detected by methylation analysis do not completely overlap with those detected by cytology. In fact, methylation analysis tend to preferably detect cervical cancers and advanced high-grade precursor lesions, whereas cytology is relatively more competent in detect early, CIN2 lesions.\textsuperscript{45} Thus, methylation markers have the advantage of reducing the risk of missing cervical cancer and advanced high-grade precursor lesions with a high short-term progression risk for cancer.\textsuperscript{45} This concept was originally born after the observation that promoter methylation levels of two genes (i.e. $\textit{CADM1}$ and $\textit{MAL}$) increased with increasing severity and duration of CIN lesions and were extremely high in cervical cancers.\textsuperscript{46}

Further support came from subsequent studies showing that all cervical smears of women with cervical cancer were positive for DNA methylation\textsuperscript{47} (Strooper et al, submitted)(Chapter 8). Thus, according to this concept, women with a negative methylation marker test would have a low
General discussion and future perspectives

short-term progression risk for cancer, which means that short-term colposcopy is unnecessary. When these findings, especially the high sensitivity for cervical cancer, can be confirmed by other studies, methylation testing alone or as an adjunct to cytology may even provide a feasible screening strategy, particularly in low- and middle income countries were quality controlled cytological assessment is absent. Since a methylation marker only strategy is relatively new for clinicians who are currently used to cytology triage, our strategy of first choice on cervical scrapes of HPV positive women is the combination of CADM1/MAL methylation analysis and cytology (Chapter 8).

Conclusions:
Methylation marker panels are attractive candidates for triage of HPV positive women since they reduce the risk of missing cervical cancer and advanced high-grade lesions.

Methylation levels increase with a longer duration of the HPV infection and with increasing severity of cervical lesions, and are exceptionally high in cervical cancer.

As long as clinicians cannot make a difference between CIN2/3 lesions with a high- and low short-term risk of cancer, the preferred triage strategy in women with HPV positive cervical scrapes is cytology testing combined with CADM1/MAL methylation analysis.

Management of women who test HPV positive on self-sampled material
Non-morphology-based triage tests directly applicable to self-sampled material will probably optimize screening of non-attendees. Previously it was shown that DNA methylation analysis of certain tumor suppressor genes can be used as triage tool both on cervical smears and on cervico-vaginal lavage material of HPV positive women for the detection of CIN2+. In this thesis (Chapter 6), we showed in a randomised controlled trial that methylation marker analysis of the bi-marker panel MAL/miR-124-2, as direct triage test on HPV positive self-collected cervico-vaginal lavage material, was as sensitive as cytology triage on an additional physician-taken cervical smear for the detection of CIN2+. Moreover, we showed a better compliance of HPV positive women and shorter time to CIN2+ diagnosis was observed with methylation marker triage, at the cost of a 1.9 times higher referral rate to colposcopy. Since the referral rate with the current threshold of the methylation marker panel was significantly higher than with cytology, we subsequently searched for the most optimal threshold for methylation test positivity (Chapter 7). However, it turns out that by increasing threshold for positivity, sensitivity of the methylation marker triage test decreased substantially.

Another non-morphology triage test that performed reasonably well when applied directly on self-sampled material was HPV16/18 genotyping. It has been estimated that HPV16 and HPV18 cause approximately 70% of cervical cancers worldwide. Therefore, sole HPV16/18 genotyping as triage test in HPV positive women does not detect those (pre)cancerous lesions associated with non-HPV16/18. By combining HPV16/18 genotyping with methylation marker testing a full-molecular triage strategy in women with an HPV positive self-sampled specimen
can be revealed. This strategy could reach a similar sensitivity with a higher methylation marker threshold for positivity as with sole methylation marker testing, but with a higher specificity for CIN3+. The combined MAL/mir124-2 and HPV16/18 genotyping strategy is our triage strategy of first choice on self-sampled material of HPV positive women as it reveals acceptable referral rates, while combining detection of women with the highest risk for advanced CIN lesions and cancer (Chapter 7).

**Conclusions:**
Bi-marker MAL/miR-124-2 methylation analysis directly applicable on self-sampled material has shown to be as sensitive as cytology triage on a physician-taken cervical scrape as triage test in HPV positive women for CIN2+ detection.

Compared to cytology triage, direct molecular triage resulted in a shorter time to CIN2+ diagnosis without an additional visit to the physician at the cost of a higher colposcopy referral rate.

Increasing the threshold for methylation positivity combined with HPV16/18 genotyping resulted in an improved full-molecular triage strategy by an increased specificity.

The preferred triage strategy on self-sampled material of HPV positive women is a combination of MAL/miR124-2 and HPV16/18 genotyping.

**FUTURE PERSPECTIVES**

As already indicated before, in 2016 primary HPV screening will be implemented in the Netherlands and HPV self-sampling will be offered to non-attendees of screening. For a successful implementation of HPV screening it is essential that women, general practitioners and gynaecologists are educated about the causal relationship between HPV infection and cervical cancer. Moreover, for HPV self-sampling, it is important to arrange the logistics with regard to delivery of the self-sampling device. Screening organisations, physicians and laboratories have to collaborate to increase the chance of success by implementing HPV self-sampling as screening tool for non-attendees. Together with the replacement of cytology based screening by primary HPV testing and the efforts to improve coverage of the HPV vaccination programme, this will hopefully decrease the cervical cancer burden.

In keeping with the recommendation of the Health Council of the Netherlands, self-sampling will be offered on request (opt-in) to the non-attendees. However, it could be considered to propose an opt-out schedule to increase attendance since opt-out has led to higher response rates than opt-in. It is likely that in the future HPV self-sampling might also be offered as alternative to all women invited for cervical screening. In that case a more thorough study should be performed in the screening population to demonstrate that the use of a given combination of HPV test and self-sampler is clinically not inferior to HPV testing on a physician-collected cervical sample.
When self-sampling is implemented in the nationwide screening programme as screening tool in non-attendees, triage of women with HPV positive self-sampled material will be needed. In the present proposal, women who test HPV positive on their self-sampled material are advised for an additional cytology smear at the physician. However, molecular triage tests such as *MAL/miR-124-2* directly applicable to self-sampled material have gained increasing attention, and therefore full-molecular screening will likely be considered in the future. Moreover, the investigated molecular methylation marker panels *CADM1/MAL* and *MAL/miR-124-2* could possibly play a role as primary screening method since these tests have a minimal chance of missing cancers and advanced high-grade precursor lesions.

In conclusion, HPV self-sampling combined with *MAL/miR-124-2* methylation analysis (and HPV16/18 genotyping) opens the possibility to full molecular cervical cancer screening minimizing the need for a cervical smear at the physician. This can result in a more efficient way to screen non-attendees of cervical screening. Even for participants of screening, a full molecular screening strategy could be promising. With the identification of the most optimal (combination of) biomarkers that are applicable directly on self-sampled material, a safe triage strategy can be obtained for women with an HPV positive self-sample in combination with an acceptable screening burden for women and clinicians.
REFERENCES


General discussion and future perspectives


