SUMMARY

Cervical cancer is the fourth most common cancer in women worldwide with over half a million new patients each year. The highest incidence rates of cervical cancer are reported in low resource countries, while the incidence and mortality rates have decreased in countries with organized screening programmes. Efficient programmed screening leads to an earlier detection of (precursors of) cervical cancer, and better survival due to earlier treatment. Since more than half of all cervical cancers are found in women who do not participate in cervical screening, targeting these non-attendees could improve the effectiveness of cervical screening and reduce the incidence and mortality of this disease. This thesis attempts to give more insight in the clinical accuracy and acceptability of HPV self-sampling and the improvement of cervical screening among non-attendees by offering self-sampling. Moreover, several triage strategies are evaluated in women tested HPV positive on self-sampled material.

Chapter 1 provides a general introduction about cervical cancer, human papillomavirus (HPV), HPV mediated cervical carcinogenesis, the prevention of cervical cancer and the ways to improve cervical cancer screening. This latter aspect includes improving screening attendance by HPV self-sampling and the management of HPV positive women.

Cytology-based screening programmes have already shown to be effective in decreasing the incidence and mortality of cervical cancer. However, this decrease has leveled off and currently the incidence and mortality rate are stable in the Netherlands. A main reason for this stabilization is the high incidence rate among non-attendees of screening. Several studies have shown that these non-attendees can be attracted into cervical screening by offering HPV self-sampling. However, it is important to find out whether an HPV test on self-sampled material is as accurate as cytology or HPV testing on physician-taken material. Therefore, in Chapter 2 we presented a review in which we compared the accuracy of self-sampled versus physician-collected samples and described the impact of self-sampling on population attendance in cervical cancer screening. We found that high-risk HPV (further referred as HPV) testing on self-samples was at least as, if not more, sensitive for the detection of cervical intraepithelial neoplasia grade 2 or worse (CIN2+) as cytology on physician-taken cervical samples, but often less specific. Similar accuracy could be achieved by HPV testing on self- versus physician-taken material with regard to CIN2+ detection, provided that a validated combination of HPV test and self-sampler is used. Moreover, our review showed that HPV self-sampling also allows making cervical screening accessible to women in low- and middle resource countries and that self-sampling is superior to a recall invitation for cytology in attracting non-attendees into the screening programme in high resource countries.

Thus, offering self-sampling can increase screening coverage by motivating non-attendees to participate in screening. However, the performance of self-sampling can be influenced by the acceptability of the device by the users, the volume of the sampled material and choice of HPV test. Therefore, in Chapter 3, we compared an ergonomically improved, second generation lavage device with a first generation lavage device among non-responders of the screening
programme in the Netherlands. We showed that participation of these non-attendees in cervical cancer screening is probably not predominantly determined by the type of self-sampling device since replacing the first generation device by an ergonomically improved device resulted in similar clinical performance and response rates.

To evaluate whether self-sampling is a good alternative for physician-taken sampling, several different self-sampling devices have been used including cotton-swabs, brushes, tampons and lavage devices. In Chapter 4 we compared the performance of two novel self-sampling devices for high-risk HPV detection. In this study, 30,130 eligible women were randomly assigned (1:1 ratio) to receive either a brush-based cervico-vaginal self-sampling device or a second generation of a cervico-vaginal lavage device for HPV testing. We found that the brush-based device is non-inferior to the lavage based device in terms of participation rate (34.6% vs 31.9%, respectively). Moreover, similar HPV-positivity rates and CIN2+/CIN3+ rates were observed in the two groups. A questionnaire that was sent to all invitees reported a similar user comfort with both self-sampling devices.

To determine why women do not attend regular cervical screening, and for which reasons these women do or do not participate when offered a self-sampling device, we analysed questionnaires of the above mentioned self-sampling study in more detail in Chapter 5. In total, 10,166 questionnaires were returned to the laboratory of which 9,484 were returned with a self-sampled specimen and 682 without. The most frequently reported reason (32.3%) for non-attendance to the regular screening programme was that women forgot to schedule an appointment with the general practitioner. The most important reason (50.2%) to participate when offered a self-sampling device was the opportunity to sample in their own time-setting. Thus, organisational barriers were the main reasons for non-attending regular cytological screening in our study population, whereas convenience and self-control were the most important reasons for these women to use a self-sampling device when offered.

Although a higher CIN2+ sensitivity is observed with HPV testing compared to cytology, HPV testing has a lower specificity. Therefore, triage of HPV positive women is needed to prevent over-referral and overtreatment. In the Netherlands, the currently most accepted triage tool for HPV positive women is cytology. However, direct cytology triage on self-sampled material is not reliable and an additional visit to the physician is needed to identify those HPV test-positive women with clinically relevant lesions. An earlier study showed that DNA methylation of biomarker panel MAL-M1 and miR-124-2 can be used as triage test to detect CIN3+ on self-collected cervico-vaginal lavage material of women with an HPV positive self-sample. In Chapter 6, we assessed whether direct DNA methylation-based molecular triage (i.e., DNA methylation analysis of MAL and miR-124-2 genes) on self-sampled cervico-vaginal material performs non-inferior to physician-based cytology triage in detection of CIN2+. In this randomised controlled trial, 46,001 non-attendees of regular cervical screening were invited to submit a self-collected cervico-vaginal lavage sample for HPV testing. All women who submitted self-sampled material and were tested HPV-positive were randomised to either direct molecular (n=515) triage on left-over self-
collected material or cytology triage (n=509) on an additional physician-taken smear. We found similar CIN2+ detection rates in the molecular triage (17.5%) and cytology triage group (14.7%). In addition, women who received molecular triage did not need an additional visit to the physician for triage testing, showed a better compliance and shorter diagnostic track, at the cost of a higher colposcopy referral rate (i.e., 55% with molecular triage vs 29% with cytology triage).

At the studied thresholds for test positivity in the above described study, triage of HPV positive women by methylation analysis yielded a higher number of test-positives than cytology triage. In Chapter 7 we studied whether the performance of methylation testing of MAL/miR-124-2 to triage women with a HPV positive self-sampling test can be enhanced by changing the thresholds for positivity and by adding HPV16/18 genotyping. The methylation assay threshold was set at four different predefined levels which corresponded with clinical specificities for end-point CIN3+ of 50%, 60%, 70% and 80% in an earlier study. Here, we concluded that increasing the current self-sample methylation thresholds would substantially decrease the clinical sensitivity for CIN3+. However, a good performance in terms of clinical sensitivity (77.6%) and specificity (54.8%) for CIN3+ was obtained when combining methylation testing at increased thresholds with HPV16/18 genotyping. This fully molecular triage strategy results in an acceptable referral rate (49.9%), while it detects women with the highest risk of cancer because of strongly elevated methylation levels and/or HPV16/18 positivity.

In Chapter 8 we further evaluated follow-up strategies in women who tested HPV positive to identify those HPV positive women in need for direct colposcopy. Since the currently most studied triage test (i.e., cytology) is subjective with a large intra- and inter-observer variability and a suboptimal sensitivity, we combined bi-marker CADM1/MAL methylation analysis with cytology as triage test on cervical scrapes of HPV positive women. We found that higher sensitivities were reached with an acceptable colposcopy referral rate by combining cytology with bi-marker CADM1/MAL methylation analysis compared to cytology only as triage test on cervical scrapes of HPV positive women. Moreover, we discussed that cytology and methylation marker testing could have a complementary effect since these tests do not detect exactly the same lesions. The combined triage strategy is particularly attractive since it reduces the risk of missing cervical cancer and advanced high-grade lesions.

In Chapter 9, we presented a review in which we evaluated the arguments in favor of implementation of HPV testing as primary screening tool to improve cervical screening efficacy and the role for HPV self-sampling to increase screening attendance by attracting former non-attendees. We also discussed recently recommended triage strategies for HPV positive women as well as alternative objective, non-morphological triage strategies. In addition, we described that HPV testing can contribute to a more efficient post-treatment CIN2+ monitoring, permitting fewer follow-up visits.

Finally, in Chapter 10 we provided a general discussion of the results presented in this thesis and discussed future perspectives and clinical consequences.