English summary

Cervical cancer is the fourth most common cancer among women worldwide with more than 500,000 new cases each year. In many developed countries, the implementation of organized cervical screening has led to a significant decrease in the number of cervical cancer due to early diagnosis and effective treatment of cervical cancer and precancer. Traditionally, cervical screening was done via cytomorphological evaluation of cells from the transformation zone of the cervix (i.e. cytology test or Pap-smear). Cytology-based screening programmes have led to a significant decrease in cervical cancer incidence and mortality, however, this decrease seems to have levelled-off in recent years. Cytology, as a primary screening test, has two major constraints: the limited sensitivity (50-65%) of cytology for detection of high-grade cervical intraepithelial neoplasia (CIN) and the limited participation of women in cervical screening (~65%). Consequently, in order to continue the decrease in cervical cancer incidence and mortality, improvement of cervical screening was necessary.

Several studies have shown that testing for HPV provides better protection against cervical cancer and high-grade CIN than cytology testing. Consequently, the Dutch Health Council advised the Ministry of Health to replace cytology by primary HPV testing, and a new HPV-based cervical screening programme was implemented in the Netherlands in 2017.

The first part of this thesis describes research that aims to evaluate potential strategies to overcome challenges within HPV-based screening. One of the main challenges in HPV-based screening is finding the most suitable method for triage of HPV-positive women. The second part of this thesis focusses on future improvement of cervical screening. The research described in this second part evaluates the feasibility of HPV self-sampling as a primary method for cervical screening.

Chapter 1 provides a general introduction on cervical cancer and precancer, human papillomavirus (HPV) and HPV mediated cervical carcinogenesis, as well as an explanation on cervical cancer prevention, cervical screening and the recent transition from cytology-based to HPV-based cervical screening in the Netherlands.

Part 1: Challenges in HPV-based cervical screening

A substantial number of different HPV assays is available, however, the clinical performance of these assays differ significantly. An international team of experts formulated criteria for validation of HPV assays. A challenge within the new HPV-
based cervical screening programme is maintaining these criteria for validation of new HPV assays.

In chapter 2, we evaluated the clinical performance of the HPV-Risk assay for the detection of high-grade CIN, and compared its performance to that of a clinically validated and accepted comparator assay (Hybrid Capture 2 [HC2]). Results show that the HPV-Risk assay is non-inferior to the HC2 assay with respect to sensitivity for the detection of CIN grade 3 or worse (CIN3+) and CIN grade 2 or worse (CIN2+). Moreover, the HPV-Risk assay had a higher specificity for CIN3+ and CIN2+ as compared to the HC2. Consequently, according to international validation criteria, the HPV Risk assay can now be considered clinically validated and can safely be used for screening purposes.

Most HPV infections are cleared spontaneously. Only a small proportion of HPV infections persists and may eventually lead to the development of high-grade CIN. Consequently, adjunct triage testing of HPV-positive women is required to distinguish women with a transient HPV infection from those with clinically relevant cervical disease who should be referred to a gynaecologist for evaluation of the cervix via colposcopy. Within the new cervical screening programme in the Netherlands, triage of HPV-positive women is performed by cytology. However, cytology is a subjective test with a limited sensitivity. Consequently, a challenge within HPV-based cervical screening is to find a more suitable strategy for triage of HPV-positive women and developing new triage tools.

P16/Ki-67 dual-stained cytology has been proposed as a strategy to make a cytological assessment more objective, thereby improving the triage of HPV-positive women. In chapter 3, we evaluated the cross-sectional and longitudinal performance of p16/Ki-67 dual-stained cytology in a cohort of HPV-positive women with normal cytology participating in population-based cervical screening. Results show that p16/Ki-67 dual-stained cytology detects more than 70% of the underlying CIN3+ lesions. Furthermore, the 5-year cumulative CIN3+ risk for women with a negative dual-stain result is 3.3%, which is clearly below the 6.9% CIN3+ risk among HPV-positive women with normal cytology and the 4.5% CIN3+ risk among HPV-positive women with normal cytology and normal repeat-cytology. Consequently, p16/Ki-67 is a suitable method for triage of HPV-positive women with normal cytology.

Another application of p16/Ki-67 dual-stained cytology is to monitor post-treatment women for recurrent CIN (rCIN). Women treated for high-grade CIN are at risk of rCIN grade 2 or worse (rCIN2+). In chapter 4 we evaluated the performance of p16/Ki-
67 dual-stained cytology among 323 women treated for high-grade CIN. In addition, we compared the performance estimates to cytology and/or HPV testing. Results show that the sensitivity of p16/Ki-67 dual-stained cytology for rCIN2+ was non-significantly lower than that of cytology (69.2% vs. 82.1%; relative sensitivity: 0.84; 95% CI: 0.71-1.01), but significantly lower than that of HPV testing (69.2% vs. 84.6%; relative sensitivity: 0.82; 95% CI: 0.68-0.99). Specificity of p16/Ki-67 for rCIN2+ was significantly higher compared to both cytology (90.4% vs. 70.8%; relative specificity: 1.28; 95% CI: 1.19-1.37) and HPV testing (90.4% vs. 76.2%; relative specificity: 1.19; 95% CI: 1.12-1.26). When co-testing was considered, combined p16/Ki-67/HPV testing showed a rCIN2+ sensitivity comparable to cytology/HPV co-testing (87.2% vs. 89.7%; relative sensitivity: 0.97; 95% CI: 0.92-1.03), but with significantly increased specificity (74.2% vs. 58.1%; relative specificity: 1.28; 95% CI: 1.19-1.38). Therefore, when considered in combination with HPV testing, p16/Ki-67 dual-stained cytology might be an attractive approach for surveillance of women treated for high-grade CIN.

Another option for triage of HPV-positive women is repeat HPV testing. It seems sensible to re-test HPV-positive women for the presence of HPV since women who have cleared the HPV infection are no longer at risk for high-grade CIN. In chapter 5 we have evaluated the 5-year risk of HPV infection and CIN3+ in HPV-positive women with normal cytology and a negative repeat HPV test in the POBASCAM cohort. For baseline HPV-positive women with normal cytology, who had a negative repeat HPV test result after 6-18 months, the risk of having an HPV (re)infection at the next screening round was 20.4%, compared to a risk of 3.2% for baseline HPV-negatives with normal cytology. CIN3+ risks were also significantly higher for baseline HPV-positive women with a negative repeat HPV test as compared to baseline HPV-negative women (2.0% vs. 0.2%). To conclude, HPV-positive women continue to have an increased CIN3+ risk, even when the repeat HPV test is negative. Therefore, intervals in primary HPV-based screening should be determined separately for HPV-positive and HPV-negative women.

The value of the currently used triage strategies has only been evaluated with results of one HPV-based screening round. The prognostic value of these strategies may change when women are invited for a second HPV-based screening round, since the second screen consists of a larger proportion of incident HPV infections as compared to persistent HPV infections. Consequently, in chapter 6, we conducted a post-hoc analysis within the POBASCAM cohort to identify feasible strategies for triage of HPV-positive women in the second round of HPV-based screening. The analysis showed that the HPV result at the previous screen is a useful marker for risk
stratification of HPV-positive women in the second round of HPV-based screening. When combined with cytology triage, it obviates the need for repeat cytology after 6-18 months. Furthermore, the combination of cytology and HPV16/18-genotyping had the highest NPV and therefore may be preferred for reasons of safety, but the strategy combining cytology with previous screen HPV result had a lower colposcopy referral rate and is expected to lead to a lower number of unnecessary treatments. The eventual choice depends on factors such as disease burden, preferences, and available resources.

In the new HPV-based cervical screening programme, screening intervals are extended from five to 10 years for screen-negative women. Consequently, women aged 40 and older who test HPV-negative are re-invited for screening after 10 years. Currently, screening intervals of five years are maintained for HPV-negative women under the age of 40, and for HPV-positive women with a negative triage. Ideally, a triage strategy is able to predict the long-term risk of women, allowing extension of screening intervals for HPV-positive, triage negative women.

In chapter 7, we have evaluated long-term (5-year) CIN3+ risks of women with different combinations of HPV and cytology test results. Moreover, special attention was paid to risks of HPV-positive women with a negative triage test. Highest 5-year CIN3+ risks were found for HPV-positive women with borderline or mild dyskaryosis (BMD) cytology (22.2%), while HPV-positive women with normal cytology had a lower risk (7.9%). Very low 5-year CIN3+ risks were observed for HPV screen-negative women (0.09%). HPV-positive women with normal cytology and negative adjunct triage testing (either HPV16/18-genotyping or repeat cytology after 12 months), had a non-negligible 5-year CIN3+ risk. Therefore, extension of the screening interval over five years only seems possible for HPV screen-negative women.

Part 2: Future perspectives of HPV-based cervical screening

HPV testing can be performed on self-collected cervicovaginal material (HPV self-sampling), which makes it possible to offer self-sampling to women in cervical screening programmes. Studies have shown that offering HPV self-sampling to screening non-attendees increases participation rates. Consequently, several countries, including the Netherlands, have implemented HPV self-sampling for screening non-attendees.

It is generally assumed that women who do normally attend clinician-based sampling for cervical screening would also prefer self-sampling. Moreover, implementation of self-sampling as a primary screening option could make cervical screening more
cost-effective as it would greatly reduce the number of general practitioner (GP) visits. However, before HPV self-sampling can be considered as a primary screening option, non-inferiority as compared to clinician-collected HPV testing needs to be assessed.

In **chapter 8**, we present the results of the IMPROVE study, which is a randomised non-inferiority trial that aims to evaluate the clinical accuracy of HPV testing on self-collected samples within an organized screening setting. The HPV prevalence between self-collected and clinician-collected samples was similar (7.4% vs. 7.2%). The CIN2+ sensitivity and specificity of HPV testing did not differ between self-sampling and clinician-based sampling (relative sensitivity: 0.96; 95% CI: 0.90-1.03; relative specificity: 1.00; 95% CI: 0.99-1.01). For CIN3+, relative sensitivity was 0.99 (95% CI: 0.91-1.08) and relative specificity was 1.00 (95% CI: 0.99-1.01). Consequently, HPV testing on self-collected samples has similar accuracy as HPV testing on clinician-collected samples. This supports the use of HPV self-sampling as a primary screening method in routine screening.

So far, little research has been conducted with respect to experiences and preferences with regard to self-sampling among women from a routine screening population. In **chapter 9**, we evaluated experiences with HPV self-sampling among a subset of women participating in the IMPROVE study. Results show that women from a routine screening population have a positive attitude towards self-sampling but express some concerns with respect to test accuracy. The majority of women prefers self-sampling to clinician-based sampling in future screening.

**Chapter 10** concludes with a general discussion. In this discussion, we review the rationale of the new Dutch HPV-based cervical screening programme. Furthermore, we discuss how the results presented in this thesis can contribute to further improvement of HPV-based cervical screening.