Novel biomarkers for cervical screening and surveillance of women treated for cervical precancer

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Summary

Cervical cancer is the fourth most common cancer in women worldwide. A persistent infection with oncogenic HPV types has been proven the causative agent. Cervical cancer develops through a series of premalignant stages. This offers possibilities for screening and treatment before these stages develop into cervical cancer. Improving prevention of cervical cancer is a continuing process. In countries that have implemented cervical cancer screening programmes, the incidence and mortality of cervical cancer have decreased significantly.

This thesis addresses several aspects of cervical cancer prevention, involving both screening and post-treatment surveillance.

Chapter 1 gives a general introduction about the cervix, the natural history of HPV and its role in cervical carcinogenesis. Current cervical cancer prevention strategies, as well as treatment and follow-up procedures of cervical precancerous lesions are also discussed.

Part one: screening

In the cytology based screening programme in the Netherlands, approximately 2.5% of participating women have a borderline or mild dyskaryotic (BMD) test result or ASC-US (atypical squamous cells of unknown significance in the Bethesda classification). Since cytology is subjective, this cytology report is influenced considerably by the quality of cytology. Only 10-20% of women with BMD harbor CIN2+ lesions and are therefore at risk of developing cancer. This warrants, further triage testing to sort out the women at risk for cancer and to protect the women with a BMD test who do not have (pre)cancer for further medical examination

In **chapter 2**, we analyzed the cross-sectional and longitudinal performance of p16/Ki-67 dual-stained cytology, a more objective method to demonstrate abnormal cells for the detection of CIN2+/3+ in women with BMD cytology results, and compared the results with those obtained with baseline HPV testing. The data demonstrated comparable sensitivity and negative predictive value of dual-stained cytology for CIN3+, combined with a significantly higher specificity. This makes p16/Ki-67 dual-stained cytology a valuable alternative to HPV testing for triaging women with BMD cytology.

Data from clinical trials have shown that screening by HPV testing provides better protection against cervical cancer and its precursor lesions than cytology screening. Several countries are now either implementing or planning to implement HPV testing as primary screening tool for cervical cancer (e.g. Australia, the Netherlands, Italy, Sweden, New Zealand, Argentina) or undertaking evaluations (e.g. England, Norway, China).

To evaluate protection against CIN3+ provided by HPV-based screening, we assessed in **chapter 3** the 5-year cervical (pre)cancer risk of women with different combinations of HPV- and cytology test results. Special attention was paid to the CIN2+/CIN3+ risk of HPV-positive women with a negative cytology triage test, since this risk determines the length of the screening interval. In this sub study of the VUSA-Screen study (HPV DNA testing in population-based cervical screening), women were screened by HPV testing and cytology.

Women were managed on presence of HPV and/or abnormal cytology. The results show that HPV-positive women with normal cytology and a negative triage test, either repeat-cytology after 12 months or baseline HPV16/18 genotyping, have a CIN3+ risk of about 4%. Since the accepted CIN3+ risk threshold is <2%, these women must therefore be followed-up within five years.

As pointed out before most HPV infections are transient and will regress spontaneously. Therefore proper triage algorithms for HPV-positive women are required to prevent over-referral and over-treatment. Using repeat cytology as a triage test works well but the cytology report result is significantly influenced by prior knowledge of the HPV status, resulting in lower specificity for CIN2+. Therefore a more objective morphological biomarker i.e. p16/Ki-67 dual staining was evaluated.

In **chapter 4,** we retrospectively evaluated the cross-sectional and long-term predictive value of p16/Ki-67 dual-stained cytology in HPV positive, Pap-normal women and compared the results with HPV 16/18 genotyping. p16/Ki-67 dual-stained cytology had a higher 3-year sensitivity for CIN3+ compared to HPV 16/18 genotyping, and a lower specificity. The 5-year CIN3+ risk in HPV-positive women with normal cytology was 6.9%. Testing these women with p16/Ki-67 dual-stained cytology reduced this risk to 3.3% in case of a negative test result. There were no significant differences in 5-year CIN3+ risk for either women with a negative p16/Ki-67 dual-stain result compared to women with a negative HPV16/18 genotyping test result. However, for both triage strategies, repeat testing within 3 to 5 years is still necessary, since the 5-year CIN3+ risk in case of a negative p16/Ki-67 dual staining or a negative HPV16/18 genotyping test result is too high to delay follow-up to the next screening round (i.e 5 years).

Part two: post-treatment surveillance

Part two focusses on women treated for high-grade cervical disease (CIN2/3). Women treated for CIN2 or CIN3 retain an elevated risk of recurrence, or even invasive cancer for years following treatment. Therefore, women are monitored by cervical cytology at 6, 12 and 24 months after treatment in most European countries. After 3 negative consecutive smears, women return to the screening program. Recently, it has been proven

that the risk for developing CIN2+ in women after 3 consecutive negative smears is similar to combined cytology and HPV testing at 6 and 24 months. In order to determine the performance of testing for HPV, cytology and co-testing in predicting post-treatment disease, we performed a systematic review and meta-analysis in **chapter 5**. The sensitivity of HPV testing to predict CIN2+ was significantly higher compared to cytology (92% and 79%), with a similar specificity (76% and 81%). The sensitivity of co-testing was highest (95%), although specificity was lowest (67%). This review supports the inclusion of HPV testing in post-treatment monitoring protocols.

Based on the results described in Chapter 5 and several other published studies, a new post-treatment surveillance protocol is proposed in **chapter 6**. The protocol consists of combined testing with both cytology and HPV at 6 and 24 months after treatment. After 2 negative co-testing results, women should be returned to regular screening. Recently, as we suggested in our proposed protocol, the Dutch Society of Obstetrics and Gynaecology has incorporated HPV testing in post-treatment surveillance.

Post-treatment disease comprises a heterogeneous group of residual and incident lesions. To further optimize the management of women diagnosed with post-treatment disease with a more objective molecular marker, we evaluated the performance of the bi-marker CADM1/MAL methylation test for monitoring women with post-treatment disease in a prospective study with the aim to discriminate between residual and incident disease **(chapter 7)**. Recently it has been claimed that this methylation test detects CIN2/3 with a cancer-like methylation profile (advanced CIN). In contrast to incident lesions, this profile is found in lesions with a longer duration of the HPV infection (> 5 years) and also have more chromosomal aberrations. It is therefore argued that these advanced CIN have a high short-term risk for cervical cancer.

All post-treatment CIN3+ lesions which occurred in a one year follow up period had a persistent (and consequently longer existing) HPV infection and two-third of these lesions were methylation positive (including all 3 carcinomas detected after re-treatment). Additionally, all incidental post-treatment CIN2 were methylation negative. Combining baseline and longitudinal follow-up results further underlines the concept; a positive methylation status at follow-up is strongly predictive for underlying advanced CIN3+. Summarizing; results showed that post-treatment monitoring by the bi-marker CADM1/MAL-methylation assay provides high reassurance against cancer and advanced CIN.

In the general discussion in **chapter 8.1,** arguments in favor of implementation of HPV testing as a primary screening tool or as a secondary triage tool to improve cytology based cervical screening program efficacy are reviewed. In addition, recently recommended triage strategies for HPV-positive women as well as objective, non-morphological triage strategies are discussed. In **chapter 8.2,** key messages of this thesis and future prospects are described.