Chapter 7

Summary & Discussion

Literature data indicate that a substantial number of cervical carcinomas diagnosed in countries with a running, organised cervical screening programme is found in women who have not responded to an invitation for cervical screening.(1;2) Targeting these non-attending women is therefore of utmost importance to reduce the incidence of and mortality from this disease.

In this thesis we firstly evaluated the screening history of women with cervical cancer in the Netherlands in order to get a better insight into this problem in relation to age and FIGO stage. In addition, this thesis reports on the results of implementing hrHPV testing on self-sampled (cervico)-vaginal material from non-attendees of the cervical screening programme. Response rates, hrHPV prevalence, compliance to follow-up, referral to physician and treatment, and yield of high-grade CIN and cervical carcinoma were investigated in relation to age, ethnicity and screening history.

In **Chapter 2**, the adherence to the screening programme of women with cervical carcinoma was analysed in relation, age and FIGO stage of cervical cancer. We found that little more than half of the women with histologically-confirmed cervical carcinoma who were eligible for invitation for the screening programme were not screened in the last screening round, thus demonstrating that women with cervical cancer are still underscreened. Moreover, being underscreened or unscreened significantly correlated with higher cervical cancer stages, especially in older women. Advanced carcinoma or FIGO stage $\geq$2B occurred in 48.7% of poorly screened women as compared to 16.0% of screened women ($p<0.001$). Our results were in line with other studies in different settings and different populations.(1;3) Finally, in those women that were screened, we noted poor timeliness with respect to follow-up/referral strategies. Therefore, ways to increase adherence to screening and to optimise organisation aspects of the screening programme are important to improve the effectiveness of the screening programme.

In the Netherlands, several possibilities have been investigated to improve the attendance rate to screening, such as inviting women by their own GPs rather than health authorities(4;5), increased computerized support to practices and delegation of tasks to nurse practitioners.(6) However, the effect on attendance of these strategies is limited. A substantial proportion of women is still unwilling to visit their physician for making a cervical smear. Reasons for this include embarrassment, language and cultural difficulties with immigrant groups, time impediments for working women and improper individual risk assessment.(7)

Previous studies from our group have shown that offering a self-sampling device for collecting cervico-vaginal material for hrHPV testing may improve the participating rate.(8-12) A Dutch pilot study showed that by offering a self-sampling device, 34% of non-attendees are willing to participate to screening, compared to 17% of women who received a second reminder for regular screening.(9) Moreover, this pilot showed that the yield of high-grade CIN and cervical cancer (CIN2+) was higher in self-sampling responders compared to screening participants (1.67 vs. 0.97%), suggesting hrHPV testing on self-sampled cervico-vaginal specimens to be an effective alternative to protect non-attendees in the cervical screening programme.

In **Chapters 3** and **4**, we continued the evaluation of offering self-sampling for hrHPV testing to non-responders of the regular cervical screening programme in a large, population-based screening setting (i.e. regions Noord-Holland and Flevoland of the Netherlands). The studies were entitled PROHTECT (*PRotection by Offering HPV TEsting on Cervico-vaginal specimens Trial*) 1 and 2. The first study evaluated a lavage device as self-sampling method (Figure 1a as used in PROHTECT-1 study, and Figure 1b shows an updated version), and in the second study a brush was offered (Figure 2). Main outcome measures were the response rate in comparison with sending a screening reminder invitation and yield of CIN2+/CIN3+. In addition, the concordance of HPV-test results between material sampled by the general practitioner and self-sampled cervico-vaginal material was determined.

Both studies showed that response rate in the self-sampling group was significantly higher than in the control group that received a recall for regular cytology screening (PROHTECT-1: 27.5%, and PROHTECT-2: 30.8%; $p<0.001$ in both studies). The yield of CIN2+ and CIN3+ lesions in self-sampling responders did not differ between both studies (i.e. 1.3% and 1.0%, respectively, in the first study, and 1.5% and 1.0%, respectively, in the second study. The overall concordance of HPV-test results between the GP-taken smear and the self-sampled specimen was 58.7% and 68.8% in PROHTECT-1 and PROHTECT-2, respectively. However, in women with CIN2+ and CIN3+, very high concordance figures were obtained (93.8% and 95.5%, respectively). Together, these data indicate that offering self-sampling for hrHPV testing is a much better alternative for women not attending the screening programme than sending them a second screening reminder letter.

In **Chapter 5** we present the overall results of pooled analyses of both PROHTECT studies with special emphasis on ethnicity, age, and screening history. Although the self-sampling response rate was higher in native Dutch women than in immigrants (32.4% versus 21.8%, p<0.01) no marked differences were found between immigrants born in non-developed countries (21.1%) compared to those of developed countries (24.0%). Thus, the lower response rate amongst immigrants can not simply be attributed to differences in ethnicity. Moreover, the response rate was independent of age. In self-sampling responders, who had not participated in the previous round of screening, increased rates of CIN2+/CIN3+/ cervical carcinoma were found compared to women who had been screened in the last invitation round.(1.4%, 1.0%, 0.2% versus 0.9%, 0.6%, 0.03%, respectively). These rates were even higher in never screened women and were independent from ethnicity. Thus screening history appeared the main determinant for risk of high-grade CIN and cervical cancer. These results indicate that offering self-sampling is a feasible and effective method to protect non-attendees of cervical screening, irrespective of their ethnic background, and that the highest benefit of this approach can be expected for underscreened and unscreened women.

Overall, data from this thesis revealed that offering self-sampling for HPV testing to non-attendees in cervical screening, would increase the coverage by at least 5.2%. The yield of CIN2+/CIN3+ and carcinoma, found in both PROHTECT cohort studies, was significantly higher than the yield of CIN2+/CIN3+ and carcinoma among women with a smear in the last invitation round. Therefore, we can conclude that the results of the PROHTECT studies strongly support the implementation of this method for women not attending the regular screening programme.

The effect of the screening programme is, however, also dependent on the follow-up strategies, for instance for women diagnosed with high-grade CIN. Recent studies have shown that HPV type 16 confers an increased risk of high-grade CIN and cervical cancer.(13-15) Therefore, addition of hrHPV testing and genotyping to post-treatment monitoring policies of women treated for high-grade CIN may improve the effectiveness of detecting recurrent/residual disease. In **Chapter 6**, we studied whether the post-treatment CIN3 rate is increased in HPV16-positive women treated for CIN3. HPV typing was performed on a cervical scrape taken before treatment using the GP5+/6+-PCR method followed by reverse line blot assay. The results showed that post-treatment CIN3 rate was significantly increased in women with HPV16 compared to those harbouring other hrHPV types (p=0.03).

None of the other hrHPV types were associated with higher post-treatment CIN3 rates. For this reason, we advise that women treated for CIN3 lesions, with HPV16 as underlying cause, should be monitored more intensively because of their increased risk of post-treatment CIN3.

Discussion and future perspectives

For years cervical scrapes taken by health professionals did constitute the basis for preparing conventional smears or liquid based cytology samples for cervical cancer screening. During the last years several efforts have been made to evaluate whether self-collected (cervico-)vaginal material could serve as a good alternative for physician-collected cervical scrapes. Dacron- or cotton swabs, brushes and tampons or various lavage devices have been used as collection devices. Data from others and us have shown that offering self-sampling can improve screening attendance in developed countries (9;16;17)and facilitate access to cervical screening in developing regions possible.(18;19) In addition, interview surveys in which participants were asked for collection preference have shown that women prefer self- collection over physician-collection.(20-23) Time and place of sampling, privacy and ease of sampling have been mentioned as advantages of self-sampling. Thus, there is a basis for self-sampling of vaginal- or cervico-vaginal specimens in cervical cancer screening.

However, self-collected vaginal samples are not suited for accurate cytological assessment, because of lower specimen quality (low cellularity)(24) poor concordance with cytology on conventional smears taken by a physician(25;26) and much lower sensitivities for high-grade cervical disease.

Many studies have therefore focused on the use of self-samplers for HPV analysis.(22;27) Collectively, these studies have shown that self-sampling can be as efficient as physician-sampling in detecting hrHPV. Discordance in hrHPV detection rates between self- versus physician-collected samples, as has been reported in some studies, most likely reflect the use of different types of self-sampling devices (swap, brush, tampon or lavage) that will influence the cell yield, as well as different hrHPV detection methods that all have their specific features in terms of analytical sensitivity and specificity for hrHPV detection. Most importantly, however, is knowledge about the performance of hrHPV self-sampling with regard to disease outcome.

Studies that have compared hrHPV testing on self-samples with cytology on physician-obtained cervical samples have shown that hrHPV testing on self-

samplers is as sensitive or more sensitive for CIN2+ than cytology on physician-obtained cervical samplers.(25;28-33) Thus, hrHPV testing on self-collected samples is a safe alternative for cytology testing on physician-taken samples.

Despite some variations between studies, also sufficient evidence has been collected that highly concordant results can be obtained between hrHPV testing on self- and physician-sampled specimens at the level of CIN2+ detection.(18;23;25;29) This is supported by data from the non-attendees of our PROHTECT-1 study.(17) Relative to their (hrHPV plus cytology triage) screened counterparts of the same age category in the regular screening programme, non-responder women of $\leq$ 33 years demonstrated a similar CIN2+ rate (i.e. 0.8%; RR 0.81; 95% CI 0.53 – 1.21). Since women of this age had no previous screening round, this strongly suggests that the sensitivity for CIN2+ of hrHPV testing on self-samples is not inferior to that of hrHPV testing on physician-collected cervical samples. Variations in reported study results likely reflect the use of different collection devices and HPV tests and protocols Therefore, for reaching clinical equivalence in terms of detecting CIN2+ the right combination of self-sampler and validated hrHPV test is likely to be important.

Given abovementioned properties, it can be envisioned that hrHPV-testing on self-sampled specimens may have value as an alternative screening tool in regular, population-based screening and/or monitoring of women treated for CIN2+. Therefore, the time has come for an implementation study in which HPV testing on self-sampled cervico-vaginal material is offered as an alternative for HPV testing on a physician taken smear for CIN2+ detection in the regular screening programme. Also the Dutch Health Council has recently advised to perform such a study.

In addition, substantial improvements in the context of triage testing can be foreseen. Since cytomorphology on self-sampled specimens is not an option, women who tested hrHPV-positive on their self-sample are currently advised to visit a physician for a cervical smear. Application of molecular triage testing (i.e., testing for the presence of CIN2+ disease-related markers by molecular analysis) directly on the self-sampled specimens is nowadays a feasible option. A recent study presents an objective methylation marker panel (i.e., CADM1 and MAL) that was equally discriminatory for CIN3+ as cytology or cytology with HPV16/18 genotyping on physician-taken cervical smears in hrHPV-positive women.(34) The efficacy of this molecular triage strategy on self-samples is currently being evaluated against that of triage via the general practitioner in the PROHTECT-3 trial. Molecular triage on self-samples opens the possibility for complete women-friendly cervical screening using objective, non-morphological molecular methods.

Figure 1a: the self-sampling lavage device (Delphi®-Screener), used in PROHTECT-1

Figure 1b: Updated version of the Delphi® screener presently in use

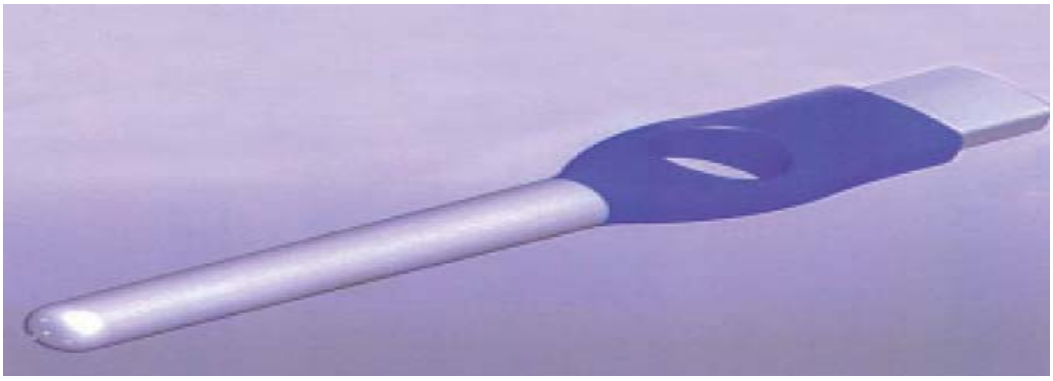

Figure 2: the self-sampling brush device (Viba-Brush®)