Triaging equivocal cytology of the cervix : identyfying women at risk for high-grade cervical lesions

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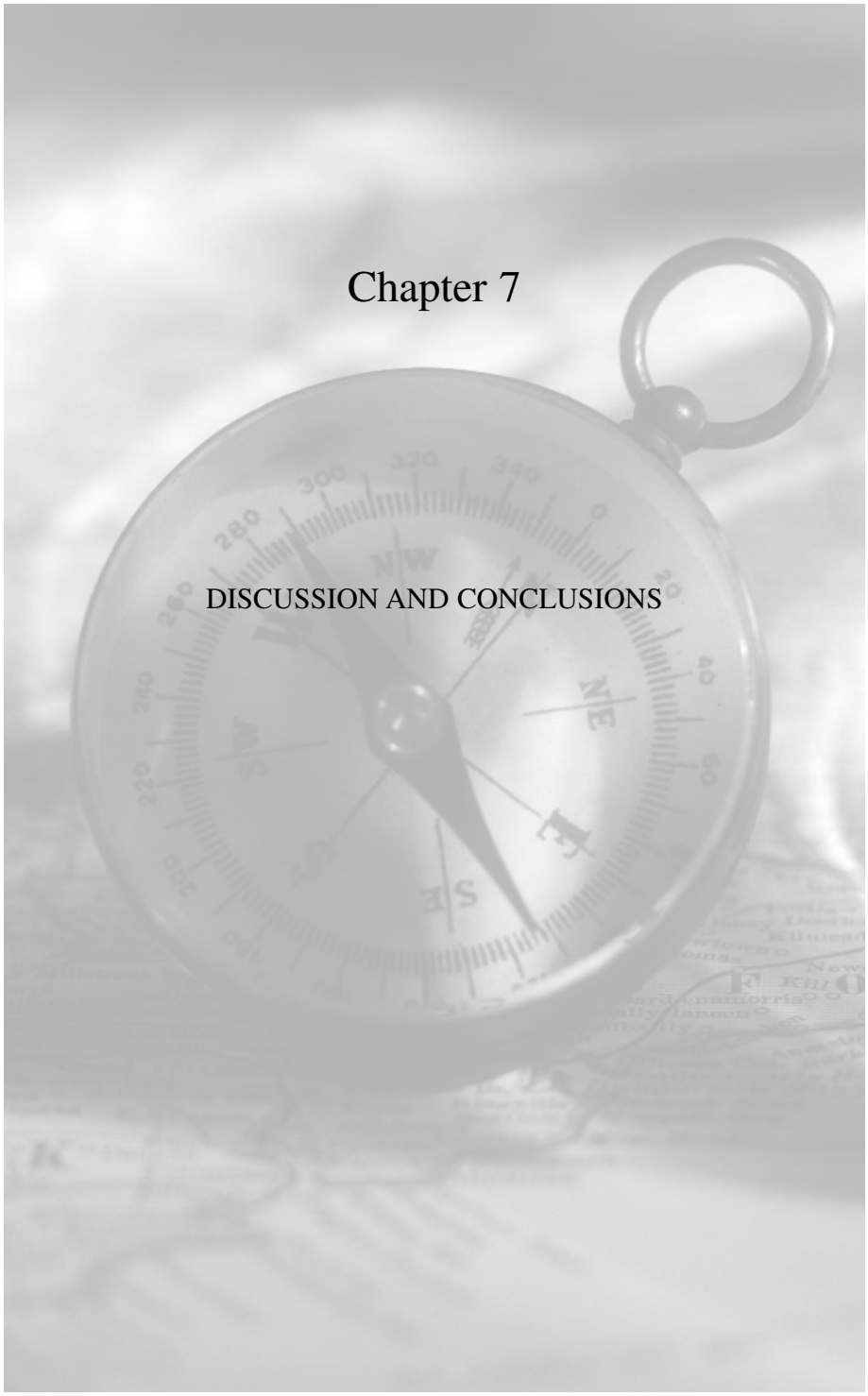


Chapter 7

DISCUSSION AND CONCLUSIONS

The majority of women with equivocal cytology (borderline dyskaryosis, ASCUS, PapII) will have trivial lesions. Some however, have significant lesions that warrant either closer surveillance or further investigation. In our group of women with borderline dyskaryosis 7% showed high-grade lesions of the cervix or cancer. Therefore we needed to identify women at risk for high-grade CIN in this category of abnormal cytology.

Smokers or users of oral contraceptives were not significantly more represented in the group of women with high-grade CIN or cancer (≥ CIN II), compared to those having normal histology or CIN I. The number of sexual partners was not a significant risk factor for detecting ≥ CIN II after adjusting this variable for HPV. We found a relatively low sensitivity (70%) and positive predictive value (33%) for colposcopy detecting ≥ CIN II in women with equivocal cytology. The specificity was 90%. Although we assessed a moderate association between each colposcopic characteristic and high-grade CIN, the negative predictive value was very high (98%). In our group of women with equivocal cytology 45% were positive for high-risk HPV. A high sensitivity (90%) for detecting ≥ CIN II and a very high negative predictive value (99%) of the Hybrid Capture II (HC II) HPV test was found. We used a ROC-curve to explore the sensitivity and specificity at different cut-off points of the HPV test for predicting high-grade CIN. The highest accuracy of the HPV test to predict high-grade CIN was found in women with low-grade cytology (borderline or mild dyskaryosis), who were over 30 years of age, and who had histology less than six months after the positive HPV test (aROC 0.74). Increasing the relative light unit cut-off value of the standard HC II HPV test (≥ 1.0 RLU) to ≥ 3.0 RLU increased the specificity of the HC II HPV test. When a higher cut-off value is used, fewer women with normal histology or CIN I will test positive, so fewer will be referred for colposcopy. Additional MIB-1 testing in HPV positive women also reduces the number of referrals for colposcopy. Although women with a viral load of ≥ 100 relative light units (RLU) showed more high-grade CIN compared to women with a viral load between ≥ 1 and 10 RLU, a considerable overlap of viral loads was seen among the grades of CIN in the women with abnormal cytology. In our study semi-quantitative HC II viral load was not a useful marker over HPV positivity alone to predict high-grade CIN. Absence (neg-neg HPV test) or clearance (pos-neg) of the human papillomavirus (HPV) showed significantly more regression to normal cytology than persistent (pos-pos) HPV or newly acquired (neg-pos) infection (odds ratio 27). Thus, absence or clearance of HPV can predict regression to normal cytology in women with equivocal cytology.

We concluded that colposcopy and human papillomavirus (HPV) testing are both important parameters in detecting high-grade cervical intraepithelial neoplasia or cancer (≥ CIN II). Biomarker Ki-67 (MIB-1 test) as a surrogate of a persistent HPV infection seems to be a potential predictor of high-grade CIN or cancer in women with equivocal cytology, but further study is needed.

Although the number of women in our studies is relatively small we suggest a HPV test in women with equivocal cytology. HPV positive women should be referred for colposcopy. Women with equivocal cytology and a negative HPV test can return to the normal screening protocol. We reviewed the literature and in the next paragraphs the results are discussed in light of supporting or contradicting our suggested management of women with equivocal cytology.

Prevalence of high-grade CIN or cancer

Many studies between 1992 and 2005 estimated the prevalence of ≥ CIN II in women with equivocal cytology[1-23]. The prevalence of ≥ CIN II ranged from 2.9 %[1] to 36.5 %[10], with a pooled mean of 10.5% (95% CI; 7.9%-13.1%)[24]. Zielinski et al.[16] published the only Dutch study. They found a prevalence of 5.6% high-grade CIN in 213 women with a single smear or two sequential smears diagnosed as borderline dyskaryosis (equivocal cytology).

Risk factors

Risk factors such as number of sexual partners (lifetime and recent), age at first sexual intercourse, use of oral contraceptives, smoking, sexually transmitted disease (i.e. clamydia trachomatis and herpes simplex virus), and parity are not consistently associated with risk of HPV infection[25,25,26,26-41]. Differences in reported findings could be attributed to methodological differences in study design, study population, specimen collection techniques, and virus detection methods.

Age and number of sexual partners, both lifetime and recent, are most consistently associated with HPV infection. A decrease in HPV infection has been observed with increasing age in populations worldwide. The highest rates occur between ages 15 and 25 and then steadily decline with increasing age, becoming steady after the age of forty. The inverse association between age and HPV prevalence appears to remain after adjustment for sexual behaviour[27,30,42-44]. However, in some populations at higher risk for cervical cancer, a second peak of HPV infection is reported among postmenopausal women[45-47].

The prevalence of HPV increases with the number of lifetime sexual partners and with the number of recent sexual partners. These findings support the sexual transmissibility of HPV infection[29,40,43,47]. Franco et al.[48] in Brazil and Kjaer et al.[49] in Denmark have shown that sexual behaviour was weakly associated with infection by low-risk HPV types, while it was a strong predictor of infection by high-risk HPV types.

Additional risk factors such as long-term use of oral contraceptives and smoking have

been extensively studied in more powerful studies, although an association with CIN and cervical cancer was less consistently found and varied by region[50,51]. In most previous studies the HPV status was not known, so the authors were unable to adequately correct for the effect of HPV.

Two recent papers provide support for the association between HPV infection and use of oral contraceptives[26,38]. Two possible mechanisms are suggested to explain the role of oral contraceptives in HPV-related cervical carcinogenesis: an increased exposure of the transformation zone to potential carcinogens and increased cell proliferation and transcription. However, a recent systematic review did not show an association between HPV infection and use of oral contraceptives and failed to provide support to the cervical ectopy hypothesis[39].

There is some evidence that smoking may be associated with the risk of cervical intraepithelial neoplasia and the susceptibility for acquisition and progression of HPV infection[32,52]. Tobacco smoking may play a role in HPV-related carcinogenesis through an immunosuppressive effect of the local immune response of the cervix, increasing the risk of acquisition, persistence, and progression of HPV infection to high-grade CIN or cervical cancer. However, the association between HPV infection and smoking is still inconclusive[27;44;53;54].

This and other studies suggest that risk factors such as oral contraceptives and smoking are probably confounding factors. Only age and number of sexual partners are consistently associated with HPV infection. The highest rates of HPV infection occur in young women (15-25 years). The prevalence of HPV infection increases with the number of sexual partners.

Colposcopy

An overview of the literature revealed that the proportion of accurate colposcopic impression was higher in cases of normal histology (61.6%) than in cases of CIN I (42.8%). In cases of histological CIN II and CIN III the percentage of accurate colposcopic impression was 59.0% and 78.3% respectively. These studies included Pap smears diagnosed as moderate dyskaryosis or more[55].

In a meta-analysis on colposcopy to predict high-grade lesions in women with cytology diagnosed as mild dyskaryosis or more, Mitchell et al. estimated a mean weighted sensitivity of 85% (range 64-99%) and a specificity of 69% (range 30-93%)[56].

Four hundred and seventy seven colposcopic images were analysed in 252 patients to determine their histopathological correlation and clinical efficiency in the diagnosing of cervical intraepithelial neoplasia (CIN). Acetowhite epithelium had the highest sensitivity (92%), the lowest specificity (25%) and a positive predictive value of 61%. Mosaicism and punctation were less sensitive (30 and 38%), but more specific (89 and

85% respectively) and had superior positive predictive values (77 and 76%)[57].
Hopman et al.[58] and Etherington et al.[59] showed a moderate (weighted) observer agreement on interpreting colposcopic images of CIN among the investigators.
In conclusion, the correlation between colposcopic characteristics and histological outcome is moderate in women with minor cytological abnormalities.

HPV test

Detection of high-risk HPV DNA is considered to be potentially useful as a triage test in women with equivocal cytology for referral for further diagnosis and treatment. There were 15 studies between 1992 and 2005 concerning the accuracy to detect high-grade CIN or cancer (≥ CIN II) in women with equivocal cytology using the high-risk HPV DNA testing probe of the Hybrid Capture II assay (HC II). All women had an index Pap smear diagnosed as borderline dyskaryosis (ASCUS or Pap II), a HPV DNA test, and all were referred to colposcopy and directed biopsies for histological verification[8-21,23]. The 15 studies mentioned above combined with our own study showed a pooled positive HPV test rate of 44.7% (Table 1). (Two Dutch studies of women with borderline dyskaryosis showed HPV positivity of 27.4% and 34.7% respectively[16,60]). The sensitivity and specificity of HC II, pooled over the 15 studies and our study, was 94% (95% CI; 92-96%) and 62% (95% CI; 56-68%) respectively[61] (Table 1).

Table 1. Accuracy to detect ≥ CIN II of triage in women with equivocal cytology using the Hybrid Capture II assay

Study	TP	FN	FP	TN	Se	Sp	PPV	NPV	Test + rate	Prevalence disease
Manos, 1999 [8]	58	7	326	582	0.892	0.641	0.151	0.988	0.395	0.067
Bergeron, 2000 [9]	10	2	38	61	0.833	0.616	0.208	0.968	0.432	0.108
Lin, 2000 [10]	27	0	12	35	1.000	0.745	0.692	1.000	0.527	0.365
Lytwyn, 2000 [11]	4	1	19	33	0.800	0.635	0.174	0.971	0.404	0.088
Shlay, 2000 [12]	14	1	47	133	0.933	0.739	0.230	0.993	0.313	0.077
Morin, 2001 [13]	17	2	88	253	0.895	0.742	0.162	0.992	0.292	0.053
Rebello, 2001 [14]	18	3	13	41	0.857	0.759	0.581	0.932	0.413	0.280
Solomon, 2001 [15]	256	11	1050	984	0.959	0.484	0.196	0.989	0.568	0.116
Zielinski, 2001 [16]	11	1	63	138	0.917	0.687	0.149	0.993	0.347	0.056
Kulasingam, 2002 [17]	23	3	115	129	0.885	0.529	0.167	0.977	0.511	0.096
Pambuccian, 2002 [18]	16	0	42	68	1.000	0.618	0.460	0.276	1.000	0.127
Pretorius, 2002 [19]	56	7	250	636	0.889	0.718	0.183	0.989	0.322	0.066
Guyot, 2003 [20]	1	0	11	11	1.000	0.500	0.083	1.000	0.522	0.043
Lonky, 2003 [21]	27	6	35	140	0.818	0.800	0.435	0.959	0.298	0.159
Wensveen, 2003 [22]	9	1	58	80	0.900	0.580	0.134	0.988	0.453	0.068
Dalla Palma, 2005 [23]	32	2	77	45	0.941	0.369	0.294	0.957	0.699	0.218
Pooled	579	47	2244	3369	0.940	0.624	0.223	0.989	0.447	0.103

The sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive value (NPV), test positivity rate and prevalence of disease (≥ CIN II) are computed from the absolute number of true-positive (TP), false-negative (FN), false-positive (FP) and true-negative (TN) results. The pooled measures at the bottom are derived from random-effect meta-analytical models (with permission of M. Arbyn, Gynecol Oncol:99:S8,2005)

In several studies the negative predictive value of HPV tests in women with equivocal cytology to predict high-grade CIN was very high (≥ 98%)[3,8,11,15].
The sensitivity was 14% (95% CI; 8-20%) higher than repeat cytology, in six studies where both (cytology alone and HPV) triage methods were used[8,11,15]. The sensitivity of the HPV test was significantly higher than repeated cytology (sensitivity ratio 1.14, 95% CI; 1.08-1.20). The pooled specificity of the HC II assay and repeat cytology were almost equal (specificity ratio 0.99, 95% CI; 0.89-1.11)[61] (Figure 1). In conclusion the hybrid capture II HPV DNA test performs better than repeat cytology as triage of equivocal cytology to detect ≥ CIN II (Figure 1).

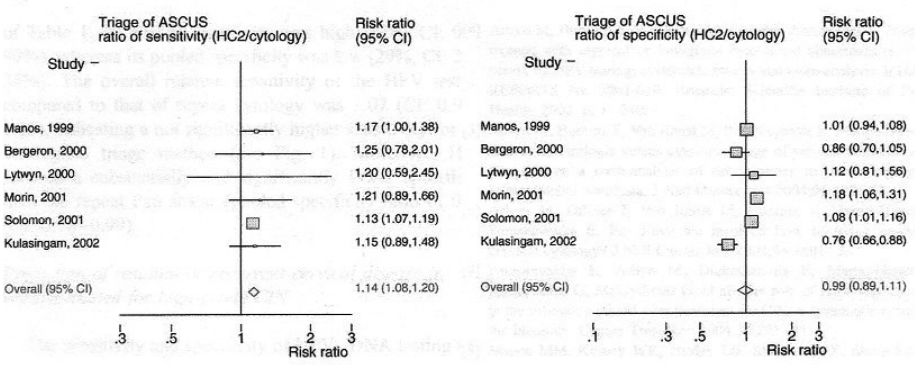


Figure 1. Ratio of sensitivity (at left) of triage using the HC II assay over the sensitivity of repeat cytology at ASCUS threshold to detect ≥ CIN II in women with equivocal cytology. At right ratio of specificity (with permision of M. Arbyn, Gynecol Oncol:99:S9,2005).

A recent study supports the use of HPV testing in triage of borderline and mildly dyskaryotic smears[62]. In 105 women with persistent borderline or mild dyskaryosis (six months interval) a "wait-and- see" policy was followed: reviewing HPV positive women with colposcopically-directed biopsy showing ≥ CIN II after 6 months, reviewing HPV negative women and HPV positive women with colposcopically-directed biopsy showing ≤ CIN I after 12 months. No progression to a high-grade CIN lesion was seen in HPV negative women. A reduction of 49% of referrals for colposcopy would have been seen if based only on the HPV test results. A "wait-and-see" period in the HPV positive women of another 6 to 12 months would have resulted in an extra 51% of HPV-tests (total 151%). This policy would also result in a further 18% (total 67%) reduction of colposcopic examinations[62]. However, a randomised clinical trial of 3348 women with only ASCUS (borderline dyskaryosis) showed that using a repeat cytology at an ASCUS threshold is as sensitive as a single enrolment HPV test but requires two follow-up visits and ultimately more colposcopic examinations (67%) than HPV triage (55.6%)[63].
Bais et al.[64] advocated that the "wait-and-see" policy increases the loss to follow-up since women may prefer a "see-and treat" policy. A randomised clinical trial of Manos

et al.[8] showed significantly (17.1%) less loss to follow-up in the HPV test group compared to the repeat cytology group. In a randomised controlled trial of cytological surveillance versus patient choice between surveillance and colposcopy in managing low-grade cytology (borderline and mild dyskaryosis) 476 women were included and followed for one year. The women were randomised either to six months cytological surveillance or were given the choice between six months cytology and immediate colposcopy. This trial indicated that having a choice did not have a favourable or unfavourable impact on anxiety or feelings of well-being. If a patient is anxious, allowing the patient to choose immediate colposcopy may be preferable because it will improve the detection rate of underlying high-grade CIN in a group who are more likely to default[64]. In conclusion, a "wait-and-see" policy seems to increase the loss to follow-up[8,65] and does not significantly decrease the number of colposcopies[60,66].

Age

The incidence of a HPV infection varies in different age categories. In a study of Howard et al.[67] 524 women with abnormal cytology were referred for colposcopy and HPV Hybrid Capture II test. The sensitivity of the HPV test to detect ≥ CIN II was lower and the specificity was higher in women over 30 years of age compared to women of 30 years and younger. The difference in specificity was significant ($p<0.001$), but the difference in sensitivity was not statistically significant[67]. For 2198 women with ASCUS cytology in the ALTS study (ASCUS-LSIL triage study, a randomised clinical trial) the overall sensitivity of HPV testing at 1.0 pg/ml for detecting CIN III or cancer was 96.1% and varied minimally with age (range 93.9%-97.8%). HPV testing at this threshold would refer 31.2% (95% CI 28.0%-34.3%) of women aged 29 years or older for colposcopy as compared to more than 65% (95% CI; 61.6%-68.9%) of younger women[68]. Schiffman et al.[69] estimated the sensitivity and specificity of the HC-II test and the referral percentage to colposcopy in 1119 women with normal or abnormal cytology. The HPV testing performance was better at older ages where the sensitivity remained constant, the specificity increased and the number of colposcopy referrals decreased.
In conclusion, the specificity of the HPV test is better in women over 30 years of age and the number of referrals for colposcopy is less than in younger women.

Persistence or clearance of HPV infection

Women infected with high-risk HPV types have a higher rate of persistent infection and a higher rate of progression to CIN compared to those infected with low-risk HPV types [25,34,70-76]. However, persistence of high-risk HPV types is rare and a considerable part of

the HPV-positive women will not develop high-grade CIN over time[77]. The persistence of a HPV infection depends on the definition of persistence and the interval of two consecutive HPV tests. Many studies have labelled HPV infections as persistent if HPV was detected on two consecutive follow-up visits, 4-6 months apart. However, because the interval between follow-up visits varies among studies and many unknowns regarding the natural history of HPV complicate the instigation of an appropriate interval, the significance of being positive at two points becomes blurred, as does the distinction between "persistence" and "transient" infection[41,78,79]. It is not always clear whether the repeat HPV test is positive because a specific HPV type persisted, or the HPV infection was cleared and another high-risk HPV type had infected the patient. The exact duration of a HPV infection is hard to measure, because it is unknown how long the women have been infected by the time they were found to be positive at enrolment. The median duration of high-risk HPV infection may vary between 8.1 to 16.6 months [33,80,81]. According to some authors, most low and high risk cervical HPV infections are cleared within two years (70-90%)[72,80,82-84]. High-risk HPV infections tend to last longer than those of low-risk HPV types. Nobbenhuis et al. analysed the HPV clearance in 230 high-risk HPV positive women with an adequate abnormal cervical smear at baseline. The cumulative one-year incidence of high-risk clearance was 35% (95% CI 28-42)[85]. Regression rates of HPV infection and mild dyskaryosis are higher among younger women[72,83]. Only one third of women older than 30 years of age cleared their infection, compared to two thirds of those younger than 24 years. In a recent study, 274 women with abnormal cytology and HC II HPV-positive test at baseline, showed a mean clearance time for high-risk HPV DNA of approximately 11 months. The clearance of high-risk HPV type and abnormal Pap test showed a close temporal relationship, the former preceding the latter, by an interval of 1-2 months[86].

Zielinski et al.[16] found significantly more regression to normal cytology in HPV negative women compared to HPV positive women with borderline and mild dyskaryosis (low-grade cytology). In their study of 278 women with a single or two sequential smears diagnosed as low-grade cytology no progression to CIN II/III was seen in HPV negative women. Two other Dutch cohort studies that compared the effectiveness of testing high-risk human papillomavirus and repeated cervical smear also showed no clinical progression or histological CIN III in women without high-risk human papillomavirus infection[62,71,72]. In one large multicentre screening study of 11085 women no progression to high-grade CIN or cervical cancer was seen in women without HPV infection or with transient HPV infection[87].

In conclusion, regression to normal cytology was seen after clearance of the HPV infection. HPV negative women did not develop high-grade CIN during follow-up. This supports the safety of the management of keeping HPV negative women in the population screening program, instead of referring them for colposcopy.

Viral Load of HC II HPV test as surrogate of persistent infection

Persistence of infection with high-risk HPV types is the most important factor for progression to high-grade CIN or cancer. Viral load is suggested as a risk factor for persistent infection[88,89]. However, the evidence is still inconclusive and its value as a prognostic marker of persistent infection or progressive disease remains unknown [76,90,91]. Brisson et al.[92] observed that high-viral load was associated with persistence of HPV infection. In several studies using a real-time quantitative PCR test a high viral load was associated with high-grade CIN[75,93-95]. Other studies suggest that semi-quantitative Hybrid Capture (HC) II viral load may not be an adequate predictor of cervical cancer [96-100]. However, some studies showed a dose-response relationship between semi-quantitative HC II viral load and high-grade CIN[101-103].

Results of viral load studies are difficult to compare, because of different study design, different population and different HPV tests (real-time quantitative PCR and semi-quantitative HC II/ PCR) that were used. The real time quantitative PCR HPV test determines the number of copies per cell to measure viral load, while semi-quantitative HC II depends on the number of exfoliated cells in a sample. The semi-quantitative HC II test cannot distinguish between infections with single or multiple types of HPV. In a recent study viral load values appeared to be lower for multiple HPV infections than single type infections. No significant association was seen between multiple HPV types and cytological outcome[104]. Moberg et al.[105] showed that quantitative PCR high viral loads of HPV 16 and 18/45 increased the risk of developing cervical cancer. Thus, HPV viral load is a type-dependent risk marker for cervical cancer.

Lorincz et al.[96] found a large overlap of semi-quantitative viral load among the grades of CIN in 2941 of 20810 women in a cohort with a positive HC II test and satisfactory baseline Pap smears. Castle et al. showed that semi-quantitative Hybrid Capture II viral load can not distinguish between $\geq$ CIN II and $\leq$ CIN II (aROC 0.57)[100]. However, a higher cut-off value (2.36 or 3.76) for the relative light unit ratio of the Hybrid Capture II HPV test may improve the management of low-grade cytology[100,106]. Nevertheless, one study of 2271 women with abnormal cytology did not recommend the use of a higher relative light unit cut-off (i.e. higher viral load), because it significantly increased the number of cases of non-detected $\geq$ CIN II: 2.4 % for cases with < 1 RLU compared to 21.7%-28.4% for cases between 2 and 5 RLU[107].

In conclusion, HC II viral load seems not to be a useful marker over HPV positivity ($\geq$ 1 RLU) alone to identify women at risk for high-grade lesions of the cervix. A higher relative light unit cut-off value of the HC II HPV test in women with low-grade cytology will reduce the number of colposcopies. However, more high-grade CIN will not be detected and the clinical usefulness is still uncertain. The importance in clinical practice of real-time quantitative PCR HPV type specific viral load to predict high-grade CIN has to be further investigated.

Biomarker Ki-67 as a surrogate of persistent HPV infection

Women with persistent high-risk HPV infection are at risk for progression to high-grade cervical lesions[77,108]. Cervical dysplasia is biologically characterised by an intrinsic proliferation of basal and parabasal cells. Ki-67 antigen is expressed in the nuclei of proliferating cells and is recognised by MIB-1 monoclonal antibody[109]. Only two relatively small studies suggest that the MIB-1-test is a significant predictor of high-grade CIN in women with abnormal Pap smears[110,111].

Because of the relatively low specificity of the HPV test (62%), women with a positive HPV test and equivocal cytology will be referred for colposcopy without having an underlying high-grade cervical lesion. Strategies that focus on identifying HPV persistence rather than prevalent infection may provide greater specificity without compromising sensitivity. Recent work has suggested that biomarkers could improve the accuracy and cost-effectiveness of cervical screening. Among the markers examined are Ki-67[112], PCNA, p16INK4[113,114], and cyclins[112], which are not usually expressed above the basal or parabasal layers in normal cervical epithelium. An important cause of their increased expression in CIN is HPV infection and the expression of viral oncogenes such as E6 and E7.

We expect that the high number of colposcopies in HPV positive women with equivocal cytology can be reduced by adding a MIB-1 test in HPV positive women. Larger studies are needed to estimate the accuracy of the MIB-1 test in women with equivocal cytology.

Cost effectiveness of HPV DNA testing in triage of equivocal cytology

The ASCUS and LSIL Triage study (ALTS) is a National Cancer Institute (NCI) sponsored multicentre randomised trial that was designed to evaluate three management strategies for detection of CIN III or cervical cancer (≥ CIN III). Immediate colposcopy, cytological follow-up, and HPV testing as methods of ASCUS triage were evaluated. A previous analysis of the ALTS data comparing the performance of these three management strategies in referring women with an initial ASCUS to colposcopy showed that repeat cytology examinations at an ASCUS threshold were as sensitive as HPV DNA testing for detecting ≥ CIN III but would require two follow-up visits[115], suggesting the possibility of higher costs for the same level of disease detection and more loss to follow-up.

Triage of women with ASCUS cytology based on a positive HPV DNA test detected more ≥ CIN III cases and was less costly than immediate colposcopy or conservative management with up to three repeat cytology visits with ≥ high-grade SIL as the threshold for referral to colposcopy[116]. In women with ASCUS cytology and over 30

years of age, HPV DNA testing was less expensive than immediate colposcopy based on either two or three repeat cytology tests, and more effective in detecting ≥ CIN III[116]. The intermediate outcome (≥ CIN III) and the short-term follow up (2 years) are limitations of this study.

The number of countries who include the use of HPV DNA testing in their guidelines for the triage of women with equivocal cytology increases[117]. At least two analyses have been conducted to explore the potential cost-effectiveness of the recommendation to refer only those women with ASCUS and a positive high-risk HPV test to colposcopy [65,118]. However, both analyses have extrapolated short-term outcomes of natural history of HPV infection and CIN to long term measures. They have relied on secondary data to estimate these measures and have made several assumptions in the absence of primary data. For example, both Kim et al.[118] and Maxwell et al.[65] assumed colposcopy and biopsy to be 100% sensitive in the absence of actual data on sensitivity. However, these analyses support similar conclusions about the favourability of HPV DNA testing as a triage strategy for ASCUS. The use of HPV triage consistently results in fewer visits, less loss to follow-up, fewer cancer cases, and fewer cancer deaths than liquid-based cytology alone at 1, 2, or 3 years screening intervals[65]. The use of HPV testing for triage of ASCUS smears is both less expensive and slightly more effective than liquid-based cytology alone at every interval[65].

Legood et al.[119] compared the lifetime effects, costs, and cost effectiveness of using cytology alone with using combined cytology and triage on the basis of HPV testing with low-grade cytology (i.e. borderline and mild dyskaryosis) in the United Kingdom (UK). In the UK, women with low-grade cytology are recalled for repeat smears every six months and only return to routine screening intervals after three consecutive negative test results. Since no long term follow-up data were available, a mathematical model was used to estimate the lifetime effects, costs, and cost effectiveness. In the publications of Kim et al.[118,120] and Maxwell et al.[65] a Markov model that simulates the natural history of HPV infection and cervical carcinogenesis was used[121]. In this model, a cohort of women aged 18-85 is simulated. The probability of acquiring a HPV infection, persistence of the infection, progression to invasive cervical cancer or clearance of a HPV infection, death from other causes, death from cervical cancer, and hysterectomy for noncervical neoplasia-related causes are variables in the model[121]. Compared with screening using conventional cytology, the next most cost effective strategy seems to be combined liquid-based cytology and HPV testing to prioritise women aged 35 or more with low-grade cytology for immediate referral to colposcopy. Although triage with liquid-based cytology alone is cheaper, it also seems less effective and has a higher cost effectiveness ratio[119]. Using HPV testing to triage women with low-grade cytology is more expensive than repeat cytology but saves slightly more lives. This gain in life expectancy is related both to referring women earlier to colposcopy and to minimising loss to follow up after the initial low-grade cytology.

Testing for HPV to manage all women with low-grade cytology (i.e. borderline or mild dyskaryosis) seems to be cost effective. The predicted increase in lifetime colposcopies, however, deserves careful consideration. Moss et al. found an increased percentage (15 to 44%) of colposcopy referrals in 3797 women with borderline dyskaryosis, when HPV triage was compared with repeated Pap smear strategy[122]. However, these doubled colposcopy rates in women aged 35-64 seem to contradict those observed in the Netherlands. Colposcopy referral rates were computed from data collected in the Dutch POBASCAM screening trial[60]. POBASCAM is a population randomised controlled trial for implementation of high-risk HPV testing in cervical screening of women between 30 and 60 years of age. The efficiency of combined HPV and cytological testing (intervention group) to detect underlying CIN III or cancer is compared to cytological testing alone (control group). In the control group 18.9% of women with borderline dyskaryosis (equivocal cytology) were eventually referred for colposcopy compared to 24.3% of women who were tested for HPV after 6 and 18 months. After correction for loss to follow-up an adjusted colposcopy rate of 29.3% for the control group was estimated. High-risk HPV testing can be implemented in triage policies for women with equivocal cytology in such a way that the number of repeat smears is markedly reduced without the cost of an increase in colposcopy[60,66,123]. Furthermore, the ALTS study showed that repeat cytology is sensitive at an ASCUS referral threshold but requires two follow-up visits and ultimately more colposcopic examinations than HPV triage: 67.1 % referrals for repeat cytology compared to 55.6% for HPV triage strategy[115]. In HPV positive women aged 29 years or older the referral rate to colposcopy even decreased to 31%[124].

In the Netherlands Berkhof et al.[123] used a natural history model for high-risk HPV infection, CIN, and cervical cancer to investigate the cost-effectiveness of adding a high-risk HPV test in women with low-grade cytology (borderline and mild dyskaryosis = BMD). The current strategy of repeat cytological testing at 6 and 18 months after BMD was compared to strategies with adjunct high-risk HPV testing. Calculations were done for both conventional and liquid-based cytology. For conventional cytology an adjunct HPV test always increased the negative predictive value compared to repeat cytological testing. The largest cervical cancer reduction rate was seen when women with a HPV-positive smear at baseline (recall of women with BMD for a HPV test within 1 month) were immediately referred for colposcopy. The number of colposcopies slightly increased compared to classical conventional cytology strategy, 28.5% versus 27.7%. The lifetime costs increased 0.2 euro per woman when the decision of colposcopy referral was based on the baseline HPV positive smear. In comparison to the classical strategy the annual diagnosis costs decreased by about 350.000 euros for the baseline ($\leq$ 1 month) HPV strategy. When liquid-based cytology was used instead of conventional cytology, the reduction in cervical cancer incidence further increased. The maximum reduction was obtained when women with a HPV positive BMD smear were

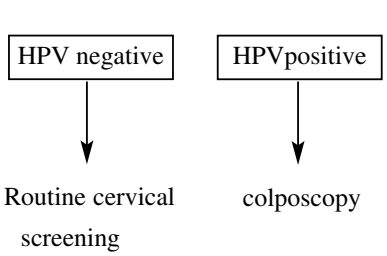
immediately referred for colposcopy. The strategy with liquid-based cytology was more effective than strategies with conventional cytology, but annual diagnosing costs were 5 million euros higher. For the HPV test at baseline cytology the difference in lifetime costs was about 31 euros per woman (317 vs. 286 euros). However, if liquid-based cytology was used, an immediate HPV test in women with BMD saved 780.000 euros compared to liquid-based repeat cytology. The number of referrals for colposcopy was slightly less in an immediate HPV test strategy (30%) compared to the repeat liquid-based cytology strategy (32.6%). In combination with conventional cytology a HPV strategy has the same overall life expectancy as the classical repeat cytology in women with low-grade cytology. An immediate HPV test at baseline low-grade cytology has equal lifetime costs, while HPV testing after 6 or 12 months has slightly lower lifetime costs. In combination with liquid-based cytology an immediate HPV test has lower lifetime costs and a higher overall life expectancy than the liquid-based cytological testing alone. Immediate HPV testing seems to be cost-effective in triaging women with low-grade conventional or liquid-based cytology[123].
In conclusion HPV DNA testing for triage equivocal cytology has a higher sensitivity than cytology alone and is more cost-effective. A HPV test instead of repeat cytology should be recommended in women with equivocal cytology. This policy results in fewer repeat Pap smears, fewer loss to follow-up and probably in a slight increase of colposcopic examinations.

Final recommendations

Currently a repeat Pap smear is advised after 6 months in women with equivocal cytology in the Netherlands. However, the Hybrid Capture II HPV DNA test performs better than repeat cytology as triage of equivocal cytology to detect high-grade CIN or cervical cancer. The accuracy of the HPV test is better in women over 30 years of age. In the Netherlands women between 30 and 60 years of age are screened for cervical cancer. For these women with a Pap smear diagnosed as borderline dyskaryosis we recommend a HPV test instead of a repeat Pap smear after 6 months.
In women with equivocal cytology the regression to normal cytology is significantly higher in HPV negative women than in HPV positive women. HPV positive women show significantly more high-grade intra-epithelial lesions than HPV negative women. The overall number of colposcopies after a long-term follow-up in women with equivocal cytology does not significantly differ between immediate HPV testing and repeat Pap smear strategy. The negative predictive value of the HPV test to predict high-grade CIN in women with equivocal cytology is very high.
We suggest an immediate HPV test in women between 30 and 60 years of age with

equivocal cytology. HPV positive women should be referred for colposcopy. When colposcopy reveals CIN I or less, the HPV test should be repeated after 18 months (this recommendation is based on literature findings mentioned in chapter 7). A four quadrant CIN II lesion, CIN III, or cervical carcinoma should be treated surgically. If a small CIN II lesion is present in a younger woman with pregnancy wish no immediate surgery is required. Follow-up can consist of repeat HPV test after 6 and 12 months. The above mentioned policy will result in an initial increase in colposcopy referrals, but in the long term the workload is expected to decrease, as HPV negative women with equivocal cytology return to routine screening. The policy will result in fewer losses to follow up. Strategies that focus on identifying HPV persistence rather than prevalence of infection may provide greater specificity without compromising sensitivity. The accuracy of real-time quantitative PCR viral load of HPV and other biomarkers (Ki-67/P16INK4) as surrogate markers of persistent HPV infection to predict high-grade CIN in women with equivocal cytology has to be further investigated.

Flow chart for equivocal cytology



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