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Chapter 10: New dimensions in cervical cancer screening

Jack Cuzick [a,*], Marie Helene Mayrand [b], Guglielmo Ronco [c], Peter Snijders [d], Jane Wardle [e]

[a] *Cancer Research UK Centre for Epidemiology, Mathematics and Statistics, Wolfson Institute of Preventive Medicine, Charterhouse Square, London EC1M 6BQ, United Kingdom*
[b] *Division of Cancer Epidemiology, Departments of Oncology and Epidemiology and Biostatistics, McGill University, Montreal, Canada*
[c] *Unit of Cancer Epidemiology, Centro per la Prevenzione Oncologia Piemonte, Torino, Italy*
[d] *Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands*
[e] *Department of Epidemiology and Public Health, University College London, London, UK*

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Abstract

Human papillomavirus (HPV) testing has clearly demonstrated a higher sensitivity but somewhat lower specificity than cytology. However, there are still issues regarding how best to use it in primary screening. In countries where cytology is of good quality, the most interesting possibility for primary screening is to use HPV testing as the sole screening modality with cytology reserved for triage of HPV-positive women. In countries with a less established infrastructure, however, use of HPV alone would also be attractive, although rapid, simple tests followed by immediate treatment are needed to minimize the number of visits and make best use of limited resources. Several approaches to deal with the lower specificity of HPV testing are also examined. These include HPV typing with a different management strategy for HPV-16 and -18/45, use of viral load to exclude infections unlikely to be associated with ≥CIN-2, and markers of proliferative lesions such as p16 and mRNA or cell-cycle markers such as cdc6 or the mcm5 proteins. Micro-array studies offer the prospect of discovering new, better DNA- or RNA-based diagnostics. The fact that HPV is a sexually transmitted infection may lead to anxiety and concerns about sexual relationships and these issues are also discussed. Ongoing HPV studies are identified and briefly reviewed.

Keywords: HPV testing; Cervical screening; Psychosocial issues; Viral load; HPV typing; p16; Cell cycle markers; Proliferation markers

1. Psychosocial aspects of HPV testing

The discovery of the viral aetiology of cervical cancer and the development of tests to detect HPV-DNA in cervical cells have significant implications for strategies to prevent cervical cancer. They also have implications for health education and quality of life. Awareness that a sexually-transmitted infection (STI) is the causal agent in cervical cancer could affect attitudes towards the disease, and care is needed to minimise any reduction in screening coverage that could come from this association with STIs. Inclusion of HPV testing into cervical screening programmes means that women will need to understand the role of HPV infection if they are to make informed choices. The high prevalence of HPV infection means that large numbers of women will receive positive HPV results and will therefore need advice and support. Promoting understanding of HPV without creating anxiety will be a challenge for psychosocial researchers.

1.1. Public awareness of HPV

At present the public is largely unaware of HPV or its role in cervical cancer, although there is recognition of a link between cervical cancer and sexual behaviour. A population-based survey in the UK in 1999 found that fewer than 30% of people identified "infection" as a possible cause of cervical cancer, with almost none mentioning HPV [1]. In a group of relatively well-educated women attending a "well-woman" service that offered cervical screening, only 30% said they

* Corresponding author. Tel.: +44 207 014 0226; fax: +44 207 014 0269.
E-mail address: jack.cuzick@cancer.org.uk (J. Cuzick).

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had heard of HPV, and fewer than half of them were aware of the link with cervical cancer [2]. Similar results have been reported from North America [3].

1.2. Reactions to being informed about HPV

Qualitative studies with women from a range of backgrounds have explored reactions to receiving information about HPV. The information typically includes the name of the virus, the mode of transmission, the prevalence of transient infection, and the associations with cervical abnormalities and cervical cancer. Most women are astonished by the information and many are shocked that they did not know before. Women's reactions to hearing about the test include confusion and anxiety about the association with STIs as well as issues of fidelity and trust in relationships [4,5]. Anhang et al. [4] identified confusion about the relationship between Pap testing and HPV testing, and uncertainty about the level of risk. Women from some ethnic and religious backgrounds express fears that community leaders could be less supportive of cervical screening if they were aware of the link with sexual transmission [5]. The association between HPV and genital warts can compound women's worry about infection if warts already carry negative connotations. Most women outside of stable relationships express concern about preventing infection in the future, either for themselves or for their partners, and the message that condoms are not fully protective can be confusing because it appears to contradict other "safe sex" messages.

Interestingly, at least in the context of discussion of self-testing, most women who have taken part in focus groups express the wish to know their HPV status [5]. Fears that women will be reluctant to take part in testing when they know about the HPV connection may not be realised if clear information is made widely available.

1.3. Reactions to HPV test results

When women are given HPV test results, either in the context of routine care or a trial, this is often the first time they have heard of HPV. Qualitative studies indicate that women receiving positive results report feeling anxious, concerned about their sexual relationships, worried about their future health, and apprehensive about disclosing their results to friends and family [6]. Quantitative results confirm these observations, finding increases in general anxiety and concern about health and relationships in women with positive HPV test results that are greater than the emotional reactions to a positive Pap test result [7].

Because HPV infection can persist, an increasing number of women in cervical screening programmes will go on to have a second HPV test. In the context of a randomized trial of HPV testing in primary cervical screening (ARTISTIC), women who were re-tested after a year were interviewed. Although they had been worried about their test result on the first occasion, most of them were able to forget it in the intervening months. If the second test was negative, the women were naturally relieved. However, if the test was positive, they were often extremely worried and many described overcoming their reluctance to disclose their test result to get support from family members [8]. The women in this sample were given the choice of further repeat testing after a year or immediate colposcopy; the majority chose colposcopy. This probably reflects their greater anxiety about HPV results compared with receiving mildly abnormal cervical screening results, where immediate colposcopy and repeat testing were more or less equally selected.

1.4. Need for information

All the qualitative studies in the field find that women want more information about HPV [4,6]. They also want their health providers to be well-informed about the disease in order to answer their questions without giving confusing and inconsistent information [4,6]. Health professionals' knowledge of HPV has not received much attention, but the experience of women with positive test results suggests that many have limited knowledge about HPV. Education of health professionals should therefore be a priority.

A common question when women are given information about HPV is how long scientists have known about it, and how it is possible that the public have not been made aware. In the past, there may have been some reluctance to publicise information on HPV for fear it would compromise screening participation, but nowadays information is available in association with most screening services. There have also been numerous media stories about HPV, connected both with testing and vaccination, albeit not necessarily containing the information that women need. This highlights the fact that promoting public awareness will need to be an active process.

Information about HPV is currently provided in several languages by the European consortium for cervical cancer education (ECCCE) via its website (www.eccce.org) among many others.

1.5. Conclusions

Public understanding about HPV has lagged behind the scientific and technical advances. Because HPV testing has significant social and psychological consequences, there is an urgent need for heath education. When women are tested for HPV, they want information and guidance both from their healthcare providers and through open sources. HPV information is complex, and many women remain confused after having read educational materials. Ensuring that HPV information is accessible to people at all levels of health literacy will be important. This should address many of the psychosocial issues posed by HPV testing and help ensure that women benefit from the scientific advances that will ultimately contribute to worldwide control of cervical cancer.

2. New ways to use existing technologies

2.1. HPV as the sole primary screening test

It is now abundantly clear that HPV testing is substantially more sensitive than cytology at detecting high-grade cervical intraepithelial neoplasia (CIN). These data are surveyed in Chapter 9. However, HPV testing is somewhat less specific than cytology, due primarily to the detection of transient infections that have not produced cytological changes. Basic principles suggest that in such circumstances, the more sensitive test should be applied first (i.e. HPV) and the more specific test (i.e. cytology) should then be used only for HPV-positive women to determine management. Management of HPV-positive, cytology-negative women presents a new challenge. Results from the HART (HPV in addition to routine testing) study suggest they can safely be managed by repeating the testing with both cytology and HPV after 1 year (Ronco G, personal communication, June 2006, [9]). Applying a similar protocol allowed increasing sensitivity for high-grade lesions in comparison to conventional cytology with only a small increase of false positives even in women younger than 35 years.

Women doubly negative at that time could be returned to routine screening while positives could be referred to colposcopy. This approach of using HPV as the sole primary screening modality has several advantages:

(i) HPV assays provide an automated, objective and very sensitive test. This allows for better quality control and reduces the basis for medico-legal claims.
(ii) Cytology can thus be reserved for the 6–10% of women who are HPV-positive. This facilitates high quality cytology and allows the employment of fewer, more focused cyto-screeners.
(iii) It also avoids the unnecessary triage of HPV-negative atypical squamous cell of undetermined significance (ASCUS)/low-grade squamous intraepithelial lesions (LSIL).
(iv) A longer screening interval is likely to be safe, which would improve both the cost and convenience of screening.

A possible algorithm is shown in Fig. 1, although this may need modification to satisfy local issues. In particular, issues of when to start screening and the appropriate screening interval are still controversial. Several ongoing studies are evaluating HPV testing in the context of primary screening. The main difference from previous studies is a focus on a reduction of CIN-3 in subsequent screens. These studies are listed in Table 1 and discussed more fully in Davies et al. [10].

Several of the potential advantages of HPV testing as the sole primary screening modality have only been demonstrated in cross-sectional comparisons or epidemiological studies, therefore large, simple, pragmatic trials comparing

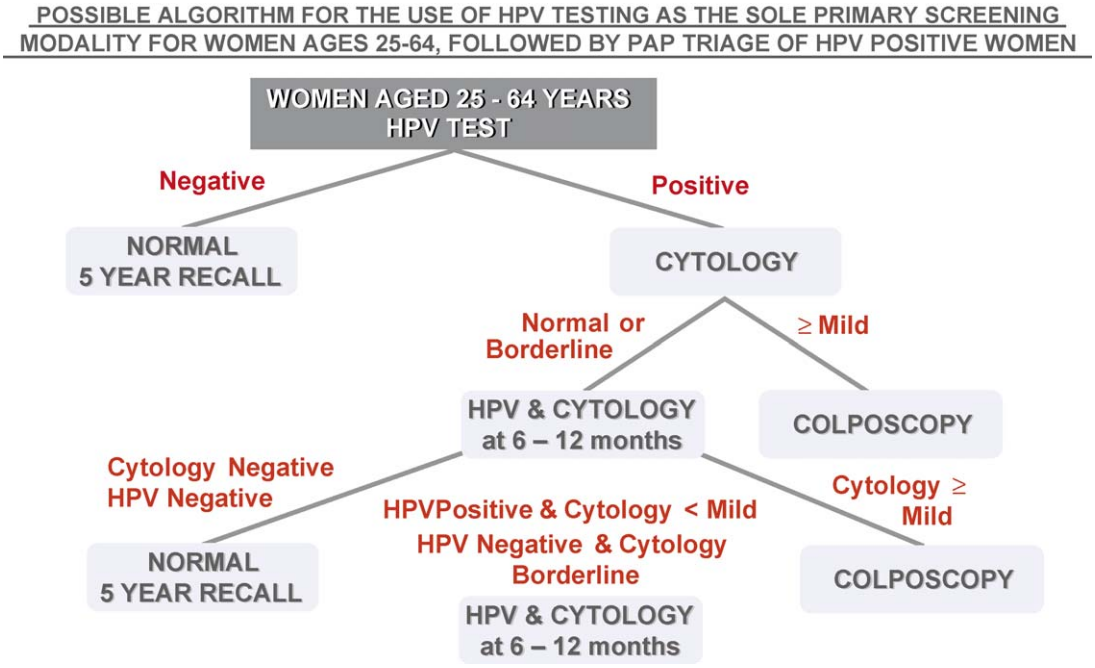

Fig. 1. Cytology abnormalities are geographically classified differently: cytology borderline (UK classification) is equivalent to atypical squamous cells of undetermined significance (Bethesda classification). Cytology mild (UK classification) is equivalent to low-grade squamous intraepithelial lesions (Bethesda classification). HPV-positive results refer to a positive result in a clinically validated high-risk HPV cocktail. Several of the options proposed in this algorithm are currently under active research. Triage options for women testing HPV-positive in the target age groups currently favor cytology, the standard of care. Other promising options under evaluation include repeat HPV testing for persistency, immediate colposcopy or the use of novel biomarkers of cancer progression, such as $p16^{ink4a}$.

Table 1
Ongoing HPV screening studies

Study	Age	Size	Intervention	Management/follow up
Dutch (POBASCAM)	30–60	44102	Pap + HPV (HPV results not revealed in control group)	HSIL: colposcopy LSIL: repeat at 6 and18 months HPV pos/cyto neg: repeat at 6 and 18 months HPV neg/cyto neg: 5 year recall
Italian (NTCC)	25–60	95000 (two phases)	(i) Pap vs. LBC + HPV (ii) Pap vs. HPV	ASCUS: colposcopy Cyto neg/HPVpos: repeat at 1 year if < 35 years; colposcopy if >35 years Cyto neg/HPV neg: 3 year recall
UK (ARTISTIC)	20–64	25000	LBC + HPV (HPV results not revealed in control group)	Cyto: mod/severe: colposcopy mild/borderline: 6 months repeat neg: 36 months recall cyto neg/HPV pos: 12 months repeat cyto neg/HPV neg: 36 months recall
Canada (CCCaST)	30–69	10154	Pap and HPV with order randomised	Cyto ≥ ASCUS or HPV+: colposcopy
Swedish (Swedescreen)	32–38	12527	Pap + HPV (HPV sample frozen unprocessed in control group)	Cyto ≥ ASCUS: colposcopy Cyto neg/HPV + : 12 months repeat
Finnish	25–65	200000 planned	Pap vs. HPV	HPV pos or cyto ≥ LSIL: colposcopy ASCUS: 6 and 12 months repeat
Mexico (MORELOS)	15–85	7868	Pap and HPV	Cyto ≥ ASCUS or HPV pos: colposcopy
UK (HART)	30–60	10358	Pap and HPV	Cyto ≥ ASCUS: colposcopy HPV pos/cyto ≤ borderline: 6 and 12 months repeat HPV pos and cyto neg: 6 and 12 months repeat HPV neg/cyto neg: 36 or 60 months recall

Pap (Papinicalou test); HSIL (high-grade squamous intraepithelial lesions); LSIL (low-grade squamous intraepithelial lesions); Cyto (cytology); LBC (liquid-based cytology).

this approach to cytology alone are needed to assess the impact of primary HPV screening on cancer incidence and mortality.

2.2. Rapid HPV tests followed immediately by treatment

Cervical cancer disproportionately affects women in developing countries. This is partly due to the fact that cytology-based screening programs are difficult to implement in such settings. Indeed, high-quality cytology requires highly trained personnel and some specialized equipment, and even when these requirements are met, Pap cytology still has limited sensitivity. Moreover, current Pap-based algorithms for prevention of cervical cancer typically entail three medical visits: one for the Pap test, one for colposcopy and biopsy and, when a pre-invasive or invasive lesion is diagnosed, a final one for treatment. In remote areas in low-resource countries, this three-visit strategy is a major hurdle to successful prevention.

The use of rapid HPV tests followed immediately by treatment offers the potential to tackle the above hurdles simultaneously. HPV tests offer the potential to improve sensitivity, and the technology may also be more suitable to low-resource settings. The Seattle-based program for appropriate technology in health (PATH), funded through the Bill and Melinda Gates foundation, initiated the START project (Screening Technologies to Advance Rapid Testing) in 2003 to develop such tests. By working with private-sector companies they aim to develop rapid, simple, accurate and affordable HPV tests. One test is a rapid batch test, based on Digene's Hybrid Capture® technology, that targets oncogenic HPVs. This technology would make it possible to test up to 96 samples in less than 2 hours. The other assay under development with Arbor Vita Corporation targets the detection of the E6 protein of oncogenic HPV types. It is a lateral flow strip that yields results in 20 min. Because HPV infection most often does not lead to neoplasia, and because E6 appears to be involved in the transformation process, this assay offers the potential to improve specificity [11].

Since both tests offer rapid results, women could eventually be screened and treated during the same visit. The possibility of self-sampling is also being explored. Preliminary model-based economic analysis has shown this strategy to be cost-effective [12]; field testing should begin in China and India in 2007.

Although promising, further issues will need to be explored before large-scale implementation can be considered. Mainly, guidelines should address the best time

to initiate screening, the interval between screening visits (if repeated), and management of positive tests. Also, the impacts of over-treatment (adverse events, risk of other STIs, impact on fertility) will need to be scrutinized if all HPV-positive women are to be treated.

2.3. Viral persistence

A substantial proportion of HPV lesions regress spontaneously over a 6–18-month period. Several studies have shown that viral persistence is necessary for CIN lesions to progress or in fact be maintained. Kjaer et al. [13] have studied 10,758 women with negative cytology aged 20–29 who developed 165 high-grade squamous intraepithelial lesions (HSIL). They showed that the odds ratio for incident high-grade lesions was 28.4-times higher (95% confidence interval, CI: 8.4–119.0) if HPV persisted compared to women in which it regressed. Other studies have documented the importance of persistence in women with small high-grade lesions [14] and low-grade abnormalities [9].

2.4. Type-specific HPV tests

Approximately 12–18 HPV types are considered to be oncogenic. However, HPV-16 and, to a lesser extent, HPV-18/45 may carry a greater risk than the others. For this reason, it may be efficient to genotype women testing positive by pooled assays, thus allowing the intensity of follow-up to be tailored. Khan et al. [15] have confirmed a sustained increase in risk of developing CIN-3 or cancer for up to 10 years after an initial positive HPV-16 result as compared to other oncogenic types. They have proposed that women who choose co-testing with Pap and HPV for primary screening, and have a normal cytology but a positive HPV test, could be triaged with a type-specific test for HPV-16. Women found to be negative on this second test could be retested with Pap and HPV after a year. However, women found to be positive would be immediately referred to colposcopy. This would preserve the high sensitivity and HPV of co-testing, while reducing the number of referrals.

Castle et al. [16] have examined the importance of identifying HPV-16 in women with an initial ASCUS or LSIL cytology. They have concluded that HPV-16-infected women with an initially equivocal or mildly abnormal cytology have a 45.5–51.6% (depending on the HPV testing method and initial smear result) risk of biopsy-confirmed CIN-2 or worse lesions within 2 years. The authors suggest that HPV-16 detection may be useful for the triage of LSIL.

2.5. Potential value of viral-load assessment of cervical smears by real-time PCR

On the basis of several studies it may be concluded that the amount of high-risk (HR) HPV-DNA (i.e. viral load) in a cervical scrape might be an important parameter to distinguish HR HPV infections that are of clinical relevance [17]. Still, most studies that performed quantitative HPV PCR methods have in common a substantial overlap of viral-load values amongst women with and without CIN-3, especially in the range of high viral loads. This precludes setting a cut-off value for CIN-3 on the basis of high viral loads. Instead, distinguishing a subset of HR HPV-positive women with clinically unimportant HPV infections on the basis of low viral loads seems more feasible. A recent study involved a comprehensive viral-load analysis of the four common HR HPV types (i.e. HPV-16, -18, -31 and -33) by type-specific quantitative (real-time) PCR on cervical scrapings of a large group of women with normal cytology participating in a population-based cervical screening trial, and of a group of women with underlying histologically confirmed CIN [18]. All women with CIN-3 had viral-load levels that were higher than those of one-third of the women with normal cytology containing the respective HR HPV type detectable by GP5+/6+-PCR. In practice, these data could lead to type-specific real-time PCR for viral-load analysis in HPV-positive women with normal cytology. Those with viral load values above the thresholds defined in this study could then be subjected to a more aggressive management. In this way, one-third of women with normal cytology that are positive for HPV-16, -18, -31 and/or -33 can be excluded from extensive follow-up in cervical screening programs because of the absence of prevalent CIN-3.

2.6. Self-sampling for HPV

In contrast to cytology, the requirements for a good sample are less rigorous for HPV testing. Several studies have evaluated the diagnostic accuracy of self-collected vaginal specimens using swabs, tampons or brushes for HPV. Ogilvie et al. [19] have produced an overview of studies which compare this to clinician-collected vaginal samples. They found an overall relative sensitivity of 74% at specificity of 84% for the self-taken sample. Although clearly not as good as a clinician-taken sample, this sensitivity compares favourably to cytology where sensitivity for CIN-2+ is typically less than 70%. Other studies have also shown good sensitivity for self-sampled HPV when histological CIN-2 is the gold standard.

These results suggest that self-sampling for HPV is a valuable screening method for women who refuse to attend for clinician-based screening and an important way of improving population coverage of screening.

3. New technologies

3.1. mRNA expression of E6/E7 transcripts

Persistent expression of the viral oncogenes E6 and E7 is a necessary step for HPV-induced carcinogenesis [20], therefore detection of E6/E7 mRNA for high-risk types may be an indicator not only of infection but of a further step in progression towards cancer. This is expected to result in increased

specificity for high-grade lesions compared to DNA detection. A high detection rate of E6 and E7 transcripts has been found in cervical cancer tissues [21] and a relationship with histological severity has been observed in cervical biopsies [22].

A kit for the detection of E6/E7 mRNA from five HPV types (16, 18, 31, 33 and 45) is commercially available (PreTect HPV-proofer®, Norchip). In a study of over 4000 women aged 30+ years [23], 3% were positive for this test while 4.4% were positive for HPV-DNA from the same five types and 4.0% had a cytology result of abnormal or unsatisfactory. Of the 14 women in this group directly referred to colposcopy and with histology-confirmed CIN-2, 12 were PreTect HPV-proofer positive and the same number were positive for DNA of the corresponding types. A potential increase in specificity could be particularly attractive in younger women, where the prevalence of HPV infection is particularly high. In women younger than 30 years, 14.5% were positive by PreTect HPV-proofer, while 20.8% were positive by type-specific PCR; only 2.8% showed cytological abnormalities [24].

These results suggest using RNA testing for triaging HPV-DNA-positive women. In a study on a small number of HPV-positive cytologically normal women, the presence of mRNA E6/E7 transcripts was less sensitive but more specific for the detection of disease at follow-up. It was also associated with persistent infection [25]. Another possible application of HPV-RNA testing is for triaging women with equivocal cytology. This could be especially interesting for LSIL cytology. Among women with ASCUS cytology, 21% were positive for PreTect HPV-proofer while 25% were positive for DNA of the same HPV types. Among women with LSIL cytology, the corresponding proportions were 30 and 50%, respectively [23].

RNA testing could also, in principle, be used as the primary screening test. Again, no direct comparison of sensitivity in cytologically normal women is available. In particular, the loss of sensitivity associated with only detecting five high-risk HPV types requires further evaluation both in primary screening and triage. A broad spectrum (15 type) mRNA test is currently under development by GenProbe.

3.2. p16

$p16^{ink4a}$ (hereafter referred to as p16) is a cyclin-dependent kinase inhibitor whose expression is negatively controlled by the pRB gene product. p16 is usually expressed at a very low level in normal cells, while it is strongly overexpressed in cervical cancer cell lines in which RB has been inactivated by the high-risk HPV E7 oncoprotein [26]. Therefore, p16 overexpression, which can be recognised by immunostaining, can be considered as a marker not only of HPV infection but also of activated expression of viral genes and of virus-induced deregulation of the cell cycle [27].

Immunostaining for p16 has been found to be associated with intraepithelial or invasive neoplasia in cervical histology specimens [28]. p16 staining of liquid-based cytology shows reasonable overall correlation with their morphological classification [29]. However, nondysplastic cells, particularly metaplastic, atrophic and endocervical cells, may display p16 immunoreactivity, therefore reducing specificity. In order to improve specificity, the application of a nuclear score to p16-positive cells has been proposed. With this approach, 1% of normal liquid-based cytology slides, 10% of LSIL and 98% of HSIL were classified as "positive" while 12, 37 and 98%, respectively, stained for p16 [30].

p16 immunostaining has been suggested as a tool for triaging women with low-grade or borderline cytology. In a study on 66 Pap smears with ASC-US or ASC-H (atypical squamous cell—cannot exclude HSIL) cytology and follow-up biopsies, 60.6% of smears stained for p16, with a 95% sensitivity for biopsy-confirmed high-grade lesions [31]. p16 could be particularly interesting among women with LSIL cytology, where triage by HPV is inefficient. p16 was observed to be positive in 58% of liquid-based cytologies from women with LSIL, while 85% were positive for Hybrid Capture-2 (HC2) [32]. Among 283 women referred for colposcopy with LSIL, persistent ASCUS or normal cytology after loop excision, 27% were both positive for high-risk HPV testing and for p16 immunostaining. They included 78.5% of biopsy-confirmed CIN-2 [33]. When using HPV testing as the primary screening test, p16 immunostaining could be applied for triaging HPV positive women in order to distinguish those who need direct referral to colposcopy from those who should better be re-tested. Preliminary results on this approach, nested in a large randomised trial, suggest in HC2-positive women, better sensitivity and specificity for ≥CIN-2 of p16 than of cytology. However, the sensitivity and specificity of p16 testing in cytology material still needs further evaluation.

3.3. Other proliferation/cell cycle markers

Based partly on the potential value of p16 to separate high-grade lesions from nonreplicating cells, other proteins involved in cell proliferation and cell-cycle regulation have been explored. The proliferation markers Ki-67 and PCNA have not shown great promise, although a full evaluation is still needed. Attention has been directed towards the DNA replication proteins cdc6 and mcm5 [34], for which initial research suggests good sensitivity and specificity. As with p16, the initial work was done on biopsy specimens, and translation of the assay for cytology is at an early stage. Recent studies confirm that expression of these proteins is important for malignant transformation and that the markers may help to separate high-grade from low-grade lesions [35] but much must still be done to establish sensitivity, and especially specificity, in a screening context.

3.4. Micro-array analysis

Newly developed genome-wide expression or comparative genomic hybridisation (CGH) micro-array analyses may

greatly advance the identification of candidate biomarkers. To date, a number of micro-array expression analyses have been performed on cervical carcinomas, and these resulted in various genes that are either overexpressed or underexpressed in cervical cancers. Genes that are differentially expressed between normal samples and carcinomas include proliferation genes, genes encoding adhesion molecules, matrix proteins and matrix metalloproteinases (MMPs). A comparison of micro-array expression profiles of high-grade CIN lesions with cervical squamous cell carcinomas (SCC) has revealed that a subset of high-grade CIN lesions has expression profiles that are more closely related to cancers than others. This suggests that markers may emerge from expression profiling studies that allow detection of progressive CIN lesions with invasive potential [36].

Alternatively, genome-wide microarray-based comparative genomic hybridisation (CGH) can be performed to detect common regions of chromosomal gains or losses from which candidate markers ultimately can be deduced. A recent micro-array CGH study on cervical SCCs and adenocarcinomas (AdCas) revealed that gains at 1q21–31, 3q12–28, and 20q11–13 and losses of 11q22–25 and 13q14–21 are rather common [37]. Further fine-mapping of chromosome 20q using multiplex ligation-dependent probe amplification showed copy-number increases for a number of genes located at 20q11–q12. Future studies will reveal whether the host-cell biomarkers identified ultimately enable a better risk stratification of HR HPV-positive women.

Disclosed potential conflict of interest

JC: Consultant (Digene Corporation, GenProbe Inc., Roche, Norchip); Research Grants (Roche)

MHM: Consultant (Merck and Co., Inc.)

JW: Consultant (GlaxoSmithKline, Sanofi-Pasteur MSD)

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