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New concepts on risk factors of HPV and novel screening strategies for cervical cancer precursors

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Summary

During the past several years, this author has been engaged in coordinating two major multicentre trials testing optional screening tools for cervical cancer (CC) in low-resource settings both in East Europe and in Latin America. These international trials include the NIS (New Independent States of the former Soviet Union) cohort (n = 3,187 women) and the LAMS (Latin American Screening) study (n = 12,114 women). In both studies, a sizeable cohort of women (887 and 1,011, respectively) have been prospectively followed-up to assess the natural history of high-risk human papillomavirus (HR-HPV) infections and the role of implicated risk factors as potential predictors of disease outcome (acquisition, persistence and clearance).

In this communication some of the key observations recently reported from the NIS and LAMS studies will be discussed, with special emphasis on i) risk factors that are still controversial (i.e., oral contraception; OC, and smoking) or not previously studied (drug addiction), on ii) reproductive factors as potential cofactors of HPV infections in cervical carcinogenesis (i.e., age at menarche, menopause), and finally on iii) the performance of different screening strategies among young and older women. Although closely related to these topics, a detailed discussion on the dynamics of HPV infections (acquisition, persistence, clearance) and their predictive factors falls outside the scope of this communication, because they have been extensively discussed in a series of original reports and in a recent review of the author in this journal.

The NIS cohort failed to establish OC as a risk factor of CC. In all future studies, the strong confounding effects from the lifestyle behavioural factors must be taken into account, while interpreting the data on OCs as potential risk factors of CC. Similarly, it now seems that the increased risk (if any) of CC among smokers seems to be attributed to the increased acquisition of HR-HPV infections, of which the smoking status is an independent predictor in a multivariate model. The same seems to apply to drug addiction as a risk factor of CC as well. The recent LAMS data show that drug abuse itself is not a risk factor of i) contracting HR-HPV infection or ii) developing high-grade CIN. Instead, drug abuse seems to be closely associated with several of the indicators of risky sexual behaviour, which predisposes the women to oncogenic HPV infections and thus indirectly contributes to the development of CIN2+ lesions.

Data from the NIS cohort clearly implicate that menarche age is not associated with increased risk of HR-HPV infection, or development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of CIN lesions or HR-HPV infections in a longitudinal setting. Another special group are postmenopausal women, recently shown to have a second peak of HR-HPV prevalence in many populations. The NIS cohort data suggest that among women who fail to eradicate their HR-HPV infection by menopause, there is i) a transition from multiple infections to single-type infections, and ii) selection of an integrated viral clone has already taken place, driving the process towards an aggressively progressing cervical disease.

Finally, these special features of HR-HPV infections among younger and older women lead us to consider, whether different screening strategies are needed for younger and older women. Consonant with other recent reports, data from the LAMS study show that conventional Pap and HC2, but not LBC and VIA, perform significantly differently among younger and older women. However, the choice of an optimal screening test for young and older women depends on whether the highest positive predictive value (PPV) (Pap test) or the best balance between sensitivity and specificity (SE/SP) (HC2) is used as the selection criteria.

Both the NIS cohort and LAMS study have significantly contributed to solving several of the open issues in the natural history of HR-HPV infections, including their risk factors, covariates necessary in cervical carcinogenesis as well as in sorting out the optional screening strategies in low-resource settings and for women in different age groups. In the long run, it is most likely that the cost-effectiveness will be the decisive factor for which screening tests will be selected. Needless to reiterate that screening for cervical cancer precursors will be mandatory until the foreseeable future, even in this emerging era of prophylactic HPV vaccination.

Key words: Human papillomavirus; Risk factors; Covariates; Oral contraception; Smoking; Drug addiction; Reproductive factors; Menarche; Menopause; Screening strategies; CIN; Cervical cancer.

Introduction

Data from carefully controlled long-term prospective follow-up studies suggest that the natural history of clinical human papillomavirus (HPV) infections in the uterine cervix is identical to that of cervical cancer precursor (CIN = intraepithelial neoplasia) lesions, with a) progression, b) persistence, and c) regression as the three main outcomes [1-

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3]. However, HPV infections have special features in their natural history that are related to the different risks of developing cervical cancer (CC) [4-6]. It seems obvious that virus type, viral load, acquisition of new (incident) infections as well as clearance of HPV or its remaining persistence, are salient features of the natural history of cervical HPV infections [2, 3, 7-11]. More light on these dynamic viral events has been provided only during the past few years, and their significance in cervical carcinogenesis is still incompletely understood. This applies equally well to the accumulation of incident HPV infections and their predictive factors [12-15] as well as to the relatively scanty data on the mechanisms of HPV clearance or persistence, reporting conflicting findings [16-21].

Since the recognition of HPV as the causal agent of CC and its precursor (CIN) lesions in the late 1970s [4, 5, 22], a substantial amount of epidemiological data has been accumulated on the potential risk factors of HPV infections, CIN, and CC [4, 5, 23-26]. It is generally accepted that oncogenic HPV types are the single most important etiological factors of CC, associated with this disease in nearly 100% of cases [4, 27, 28]. Since the first reports (in the mid 1980's) on the risk factors predisposing women to genital HPV infections [29, 30], it has become increasingly clear that several cofactors are needed to complete the causal pathway from oncogenic HPV infection to high-grade CIN and eventually to invasive CC [4, 23-31]. The role of many such potential risk factors has been revisited in the recent literature, e.g., the role of cigarette smoking, drug addiction, oral contraception (OC), and reproductive factors (menarche, menopause).

Of the potential cofactors necessary for the development of high-grade CIN and CC, those associated with reproduction have attracted increasing attention only recently [4, 26, 32-38]. Apart from the role of OC [32-39], such reproductive factors of interest as potential co-factors of HPV infections in cervical carcinogenesis include parity [32, 34, 40-43], age at first intercourse [26, 33, 35, 39, 41-44], age at first full-term delivery [33, 35, 45, 46], menopause [47, 48], and age at menarche [42, 46, 49, 50]. The role of parity and age at first intercourse are well established risk factors for HPV, whereas data are more scanty and/or controversial concerning the other listed factors.

Since the general acceptance of the concept that HR-HPV types are the single most important etiological agents of CC, it has been widely recognized that testing for HPV might offer an alternative strategy to identify women at risk for CC, at the stage when conventional Pap smear cytology is still negative or inconclusive [51-54]. This has prompted launching of a variety of guidelines and recommendations for novel strategies in CC screening during the past few years [54-58]. Data from several recent trials suggest that screening strategies optimal for women below 30-35 years of age should be different from those used to target older women [59-61]. Until now, however, these strategies have been tested almost exclusively in well resourced Western countries, where a necessary infrastructure for CC screening exists [59-62], and little data [61] are available on the feasibility of these different screening strategies among non-privileged women in low-resource settings where CC burden is the highest.

During the past several years, this author has been engaged in coordinating two major multi-centre trials testing optional screening tools in low-resource settings in both East Europe and in Latin America. These international trials include the NIS (New Independent States of the former Soviet Union) cohort (n = 3,187 women) [63] and the LAMS (Latin American Screening) study (n = 12,114 women) [64]. In both studies, a sizeable cohort of women (887 and 1,011, respectively) have been prospectively followed-up to assess the natural course of HR-HPV infections and a wide variety of implicated risk factors as potential predictors of the disease outcome (acquisition, persistence and clearance).

In this communication, some of the key recent observations from the NIS and LAMS studies will be discussed, with special emphasis on i) risk factors that are still controversial (i.e., OC, smoking) or not previously studied (drug addiction), on ii) reproductive factors as potential cofactors of HPV infections in cervical carcinogenesis (i.e., menarche, menopause), and finally on iii) the performance of different optional screening strategies among young and older women. Although closely related to these topics, a detailed discussion on the events of HPV infections (acquisition, persistence, clearance) and their regulators falls outside the scope of this communication, because it has been extensively discussed in a series of original reports [21, 65-67] and in a recent review of the author in this journal [68].

Oral contraceptives as a risk factor for HR-HPV infections and CIN

Shortly after introduction into general use, OC was implicated as a risk factor with serious health impediments, including a variety of hormone-dependent cancers [69, 70]. The first reports on possibly increased risk of CC among OC users [69-72] were followed by a large number of epidemiological studies reporting contradictory results regarding OC use as a risk factor for CC. Up to today, there are many more reports that have failed to establish any increased risk for CC associated with OC use [73-87] than those implicating that OC use increases this risk [88-96].

Oral contraceptives and HPV infections

Since the recognition of the causal link between HPV and CC [2-5, 28], increasing attention has been focused on interactions between HPV and OC use, raising the question as to whether OC is an independent risk factor of CC or whether such a reported risk is merely due to confounding effects by HR-HPV [73, 74, 77, 79, 80, 89, 90, 97-100]. In the first published report on risk factors for HPV transmission in 1984, use/non-use of contraception in general emerged

among the most significant ones [29], but we subsequently failed to establish any increased risk for HPV among OC users [26]. Since the early 1990s, a large number of studies have been published, reporting either an increased risk of HPV infection among OC users [98-100, 106], no such risk at all [26, 34, 97, 107-113], or even a protective effect of OC use on the incidence of HPV infections [114-117].

IARC multi-center case-control studies

All these data have been repeatedly reviewed by IARC experts, resulting in two separate monographs [36, 118]. In the most recent one, these experts based their evaluation on the pooled data from eight IARC multi-centre case-control studies comprising 1,561 CC patients and 1,916 controls [118, 119]. Compared with never-users, women having used OC for less than five years did not show an increased risk of CC (OR = 0.73; 95% CI, 0.52-1.03) [119]. However, OR for CC was 2.82 (95% CI, 1.46-5.42) among OC users for five to nine years, and OR = 4.03 (95% CI, 2.09-8.02) for those having used OC for > 10 years, leading the authors to conclude that long-term use of OC could be a cofactor that increases the risk of CC in women who are positive for HPV DNA. In subsequent reviews, these data were interpreted with more caution, however [120, 121], and even the WHO does not recommend any changes in the practices of using oral contraceptives [122].

Analysis of OC in the NIS cohort

As mentioned above, a cohort study testing 3,187 women for optional screening tools was conducted in three NIS of the former Soviet Union; almost 900 of these women were followed-up to assess the natural history of HPV infections [21, 63, 65-67]. In this NIS cohort study, we also analysed sexual habits and other potential risk factors of CC [123]. Using A) women with no contraception and B) those with non-hormonal contraception as controls, we recently estimated the role of OC use i) in predisposing the women to HR-HPV infections, ii) as an independent risk factor for high-grade CIN or high-grade squamous intraepithelial neoplasia (HSIL) (intermediate endpoint markers in cervical carcinogenesis), and iii) as a predictor of HPV persistence during the follow-up [124]. The key observations are discussed in some detail in the following.

Data from the NIS cohort

Interestingly, the three groups of women with different modalities of contraception were practically identical with regard to their HR-HPV prevalence, Pap smear abnormalities and CIN grades [124]. In contrast, the three groups differed significantly ($p = 0.0001$) in several important characteristics of their obstetric and gynaecological history as well as their sexual preferences. In most respects, OC users and non-OC users were alike but differed from the group of non-users of contraception, e.g., the patient category, number of abortions, age at onset of sexual activity, number of partners during the previous 24 months, STD history, casual sex partners, and history of skin and/or genital warts. Although the significant differences represented a minority of the 66 items recorded by the questionnaire [123], they included all the key variables of sexual behaviour that are known risk factors for CC and CIN. These data indicate that women with different contraceptive modalities also have a significantly different sexual behaviour [124].

When the three groups were analysed for the predictors of high-grade CIN (CIN2+) in univariate analysis, HSIL Pap smear was the only significant predictor common to all three groups, while the number of deliveries predicted CIN2 in OC users and in women with no contraception [124]. All other predictors were different in the three groups, and the list of significant predictors was most extensive for women without any contraception. When analysed separately for HPV-positive and HPV-negative women, use of OC was not a significant predictor of CIN2/3 in either group; OR = 0.98 (95% CI, 0.53-1.82) and OR = 0.92 (95% CI, 0.10-8.85), respectively. This is another indicator that the factors explaining the detection of CIN2/3 in these three groups are different [124].

When the three groups were analysed for the predictors of HR-HPV infections, many more predictors were equally strong in all three groups. Accordingly, age below 35 years, being an STD or GYN patient, and HSIL Pap test were all highly significant predictors of HR-HPV, while previous pregnancy was a significant protective factor against HR-HPV. Several other variables seemed to be significant predictors in two of the three groups, and an additional few predicted HR-HPV in only one of the groups, implicating marked differences in the sexual habits and other recorded epidemiological variables among the three groups [124].

In the next step, whether the use of OC is of any significance to the outcomes of cervical disease and HPV infections was assessed as determined by repeated Pap tests and HPV-testing with HCII [21, 63, 65-67, 124]. Importantly, all three groups were practically identical in their baseline HPV/Pap status ($p = 0.440$), and no differences could be established among the three groups as to the outcome of their cervical disease or HR-HPV infections. This suggests that the mode of contraception (or non-use of any contraception) is not a significant determinant of the outcome of cervical disease or HR-HPV infections [124].

In the whole cohort, several of the variables were highly significant ($p = 0.0001$) predictors of HR-HPV in univari-

ate analysis, but importantly, neither the mode of contraception nor hormonal contraception (use/non-use) were of any predictive value [124]. When entered in a multivariate model, only four of these variables proved to be independent significant predictors: age < 35 yrs, patient category, HSIL (all with p = 0.0001), and being a current smoker (p = 0.001). Not unexpectedly, the mode of contraception or OC use were of no predictive value in this multivariate analysis. Finally, multivariate analysis was performed to disclose the independent predictors of high-grade CIN [124]. Only two of the almost 70 variables tested [123] proved to be significant in the final regression model: (i) Patient category (protective when STD category was used as reference), and (ii) HR-HPV detection. Importantly, the two variables recording contraception were not included among those five independent predictors of high-grade CIN [124].

OC and the risk of HR-HPV and CIN: conclusions from the NIS cohort

In analysing the role of OC as risk factor for CIN, three hypotheses were tested in the NIS cohort: i) to demonstrate that sexual behaviour is indeed different among OC users, non-OC users and non-users of contraception; ii) those different habits (irrespective of OC use) are the risk factors predisposing these women to HR-HPV, development of high-grade CIN or HSIL, and also influence on the outcome of their cervical disease/HR-HPV infection, and iii) that the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis.

On the basis of the results discussed above [124], it can be concluded that these observations fully confirm the three hypotheses, while demonstrating that i) sexual behaviour is different among OC users, non-OC users, and non-users of contraception; ii) these different risk factors predispose women to HR-HPV, development of high-grade CIN or HSIL, and also influence the outcome of their cervical disease/HR-HPV infection, which is similar irrespective of their OC status, and iii) the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis. The implications of these observations are straightforward: failure to record the epidemiological data on the sexual behaviour and gynaecological and obstetric history inevitably leads to erroneous conclusions on the role of OC as an independent risk factor of CC and its precursors [124].

Smoking as a risk factor of high-risk HPV and CIN

Of the other potential cofactors necessary for the development of high-grade CIN and CC, the role of cigarette smoking has attracted increasing attention since the early 1980s [125-130]. This early literature was reviewed in 1990 by Winkelstein, leaving the role of smoking as a risk factor of CC an open issue [131]. As emphasised, most of these early studies failed to control for the residual confounding from the sexual habits [29] as explanatory factors of this smoking-CC association [129, 131, 132]. Controlling for the confounding effect of HPV in studies assessing smoking as a risk factor for CC has been particularly problematic [92, 133-135].

Recent data on smoking as a risk factor are incomplete

During the past few years, smoking as a risk factor for CIN and CC has been examined in several studies using sensitive laboratory methods for HPV detection [84, 85, 97, 135-137]. Increasing evidence from these studies suggests that smoking increases the risk of HSIL, CIN and CC [93, 138-142]. Thus, in a recent pooled analysis of eight IARC case-control studies, there was an excess risk of CC among HPV+ women for both current smokers (OR = 2.30, 95% CI, 1.31-4.04) and exsmokers (OR = 1.80, 95% CI, 0.95-3.44) [143]. In the only prospective study published so far, smoking increased the progression from CIN1 to CIN3 [144]. Similarly, smoking was significantly associated with the failure of CIN treatment [145]. Data are emerging to suggest that smoking directly interferes with the natural history of HPV, i.e., by increasing the chance for virus persistence while prolonging its clearance [19].

Analysis of smoking in the NIS cohort

In the NIS cohort, the role of cigarette smoking as a potential predictor of two intermediate endpoint markers in cervical carcinogenesis was recently analysed: i) HR-HPV infection, and ii) development of high-grade CIN [146], as well as the effect of smoking on the outcome of cervical disease and HR-HPV infections. The original questions recording the patients' smoking history included the following items: 1) Are you a regular smoker? 2) If yes, how long have you been a regular smoker? 3) How many cigarettes per day? 4) If not presently, have you ever smoked? 5) When did you stop smoking? 6) How long did you smoke regularly (yrs)? 7) How many cigarettes per day? 8) Does your sexual partner smoke? Based on these records, the cohort (n = 3,187) was stratified into three groups according to their smoking history: i) current smokers (n = 726), ii) past smokers (n = 365); and iii) never smokers (n = 2,096).

Data from the NIS cohort

Those three groups differed significantly in most of the key epidemiological variables implicated as risk factors of CIN/CC, including the prevalence of HR-HPV [146]. In fact, the list was very short for those variables which were not significantly different among the three groups. The most relevant of those non-significant variables include: a) the dis-

tribution of Pap smear abnormalities, b) detection of various CIN grades, c) mode of contraception, d) history of genital warts, and history of previous CIN. In smoking related items, the past smokers and current smokers seem to be clearly different as well. Having a sexual partner that was a smoker was significantly more frequent among current smokers.

As to the predictors of CIN2+ in univariate analysis there was no single risk factor in common for all three groups. Age below 35 years was most influential (protective) among never smokers and current smokers, but not significant in past smokers. The Hybrid Capture II (HC2) test was a significant predictor of CIN2+ only among never smokers. Not being nulliparous was a risk factor of CIN2+ only among current smokers. Of the other risk factors, previous Pap smears taken was of no significance among smokers. As compared with predictors of CIN2+, there are many more significant predictors of HR-HPV common to all three groups (or at least two of the three). The following were the risk factors common to all three groups: 1) age < 35 years, 2) patient category, and 3) HSIL in Pap tests.

Outcomes of cervical disease and HR-HPV infection in the prospective cohort of 854 women were determined by repeated Pap tests and HC2 assays. Importantly, all three groups were practically identical in their baseline HPV/Pap status ($p = 0.438$), and no differences could be established among the three groups as to the outcome of their cervical disease ($p = 0.147$) or HR-HPV infections ($p = 0.244$). These data clearly implicate that the outcome of cervical disease or HR-HPV infections are not related to the smoking status of these women [146].

In the whole cohort, several variables were highly significant ($p = 0.0001$) predictors of HR-HPV in univariate analysis. Importantly, being a current smoker and having a sexual partner as a current smoker were both significant predictors of HR-HPV. When all these highly significant predictors were entered to a in multivariate model, only five of these variables proved to be independent significant predictors: 1) age < 35 yrs, 2) patient category, 3) HSIL (all at $p = 0.0001$ level), 4) being a current smoker ($p = 0.014$), and 5) cervical erosion treated ($p = 0.035$, protective). Finally, multivariate analysis was performed to disclose the independent predictors of CIN2+ in the whole cohort. Only two of those proved to be significant in the final regression model: i) patient age < 35 years (protective) ($p = 0.0001$), and ii) HC2+ result ($p = 0.014$). Importantly, none of the smoking history/status variables were included among the independent predictors of high-grade CIN in this multivariate analysis [146].

Smoking and the risk of HR-HPV and CIN: conclusions from the NIS cohort

Taken together, this study does not provide evidence that cigarette smoking is an independent risk factor of CIN2+. Indeed, smoking status is strongly confounded by sexual habits, and few predictors of HR-HPV and CIN2+ are common to never smokers, past smokers and current smokers. There was no indication that the increase in the risk of CC is mediated by increased progression of the disease among smokers. Instead, the increased risk (if any) of CC among smokers is most likely attributed to the increased acquisition of HR-HPV infections, for which the smoking status was a significant independent predictor in a multivariate model.

Drug addiction as a potential risk factor of HR-HPV and CIN

Of the other potential cofactors contributing to the development of high-grade CIN and CC, the role of drug abuse or addiction has surprisingly attracted little attention [147-152]. Until now, all published studies have analysed only human immunodeficiency virus (HIV)-infected women who are drug addicts and/or intravenous drug abusers [147-158]. Data are unanimous in that HR-HPV infections and CIN or HSIL are significantly increased among these HIV+ intravenous drug abusers [159]. Only one recent study has addressed the risk factors of HPV infections in a cohort where drug abuse was the primary inclusion criteria [152]. In that study no controls were examined, however, of the 230 women, 24% were HIV-infected. Thus, we have no data whether drug addiction (abuse) is an independent risk factor of HR-HPV infections or CIN, when controlled for confounding effects from HIV infection or other indicators of high-risk sexual behaviour.

Analysis of drug addiction in the LAMS study

In the LAMS study [64], the role of drug addiction (abuse) was recently analysed as a potential predictor of two intermediate endpoint markers in cervical carcinogenesis: i) HR-HPV infection, and ii) development of high-grade CIN [160]. A nested case-control (1: 4) study was designed, with strict age-matching of the 109 cases (drug abusers) with 436 controls (non-abusers), and analysed using conditional logistic regression for covariates of drug abuse as well as for predictors of HR-HPV and CIN2+ in univariate and multivariate regression analyses.

Data from the LAMS study

In this age-matched case-control setting, the two groups were significantly different with regard to several known or implicated risk factors of HPV, CIN and CC [160]. Accordingly, HR-HPV infections were more prevalent (37.7%) among cases than in controls (21.9%) ($p = 0.019$). Age at first sexual intercourse was significantly ($p = 0.0001$) lower among cases. The case women were also more frequently pregnant at the time of the interview ($p = 0.022$), albeit not

different in any other pregnancy-related variables. There was a highly significant difference between the two groups in their number of lifetime sexual partners; 7.8 vs 2.8 among cases and controls, respectively. Case women had contracted with a partner who had a STD and also reported a personal history of STD significantly ($p = 0.0001$) more often than controls. Modes of contraception were dramatically different between the two groups, and use of oral contraception was more frequent among controls. The same was true with ever having a Pap smear taken ($p = 0.021$). Ever having been a smoker or being a current smoker was significantly more frequent among cases than in controls.

Conditional logistic regression analysis was conducted to assess the covariates (explanatory factors) associated with drug abuse/non-abuse (as a dependent variable) [160]. The following covariates were significantly associated with drug abuse in univariate analysis: 1) being single rather than married, 2) early onset of sexual activity, 3) being pregnant at the interview, 4) higher number of pregnancies, 5) higher number of abortions, 6) higher number of lifetime sexual partners, 7) contracted with sex partners who had a STD, 8) less frequently used OCs, 9) history of a STD, 10) more rarely a previous Pap smear taken, 11) being more often current or past smokers, with longer history of smoking and higher number of daily cigarettes smoked. When adjusted for all significant covariates in univariate analysis, only five of these variables proved to be independent covariates of drug abuse in multivariate conditional logistic regression: 1) more than five lifetime sexual partners ($p = 0.0001$), 2) ever having been an active smoker ($p = 0.0001$), 3) oral contraception (protective) ($p = 0.013$), 4) ever having had a Pap smear taken (protective) ($p = 0.027$), and 5) ever had a STD ($p = 0.041$) [160].

Not unexpectedly, several of these covariates of drug abuse were also risk factors of HR-HPV infections, when univariate and multivariate logistic regression were used to assess the predictors of HR-HPV infection. Of the multitude of factors associated with HR-HPV in univariate analysis, only three proved to be independent predictors in multivariate analysis: 1) age below 30 years ($p = 0.045$); 2) number of lifetime sexual partners > 5 ($p = 0.046$); and 3) being a current smoker ($p = 0.005$). Importantly, drug abuse itself was not a significant independent risk factor of HR-HPV infection (OR = 0.70, 95% CI, 0.31-1.58 for non-abusers), although it increased the risk in univariate analysis [160]. As anticipated, few variables predicted CIN2+ even in univariate analysis, and being a drug abuser was not one of those. Being HR-HPV positive was the only independent determinant of CIN2+ in multivariate analysis. Importantly, being a drug abuser did not increase the risk of CIN2+ in either univariate- or multivariate analysis.

Drug addiction and the risk of CIN: conclusions from the LAMS study

This was the first study addressing the importance of drug addiction as an independent risk factor of both oncogenic HPV infections and CIN2+ lesions [160]. These analyses confirm that drug abuse itself is not a risk factor of i) contracting HR-HPV infection or ii) developing CIN2+. In this matched case-control setting, being HR-HPV positive is not among the significant independent covariates of drug addiction, which is closely associated with several of the key indicators of risky sexual behaviour. The latter in turn are independent risk factors of HR-HPV infections, whereas drug addiction is clearly not. Being such a powerful surrogate marker of high-risk sexual behaviour, HR-HPV remains the only independent risk factor of CIN2+ in multivariate analyses. In simple terms, drug abuse seems to be closely associated with several of the key indicators of risk sexual behaviour, which predisposes women to oncogenic HPV infections, and thus indirectly contributes to the development of CIN2+ lesions.

Age at menarche as a risk factor of HR-HPV and CIN

Increasing attention has been recently paid to characteristics associated with reproduction as potential cofactors of high-grade CIN and CC [4, 26, 32-38]. Apart from the role of OC [32-39, 124], such reproductive factors of interest include parity [32, 34, 40-43], age at first intercourse [26, 33, 35, 39, 41-44], age at the first full-term delivery [33, 35, 45, 46], menopause [47, 48], and age at menarche [42, 46, 49, 50]. The role of parity and age at first sexual intercourse are well established risk factors of HPV, whereas the data on the other listed factors are more scanty and/or controversial.

Data on age at menarche are controversial

Until now, only a few studies have specifically addressed the significance of age at menarche as a potential cofactor of HPV in the development of CIN [50, 161, 162], and in a few others, this topic has been assessed in addition to other potential reproductive risk factors [33, 34, 49, 163]. In most of these studies, only the role of age at menarche was analysed [33, 34, 49, 163], while three studies also assessed another menarche-age-derived variable, i.e., the time from menarche to the first sexual intercourse (TMI) [161-163]. Determined from these reports, the role of age at menarche (or TMI) as risk predictors of HPV infection or CIN seems to be highly controversial [33, 34, 42, 46, 49, 50, 161-163].

Analysis of menarche age and derived variables in the NIS cohort

The NIS cohort recently analysed age at menarche as potential predictor of two intermediate endpoint markers in cervical carcinogenesis: i) HR-HPV infection, and ii) development of high-grade CIN [164]. Apart from the menarche

age itself, the role of three menarche-derived variables were further evaluated: i) time from menarche to first sexual intercourse (TMI); ii) time to first pregnancy (TMP), and iii) time to the first full-term delivery (TMD), all variables being analysed both in univariate and multivariate models.

Data from the NIS cohort

Based on the patient records, women were stratified into three groups according to their menarche age: i) menarche age < 13 years; ii) those between 13-14 years; and iii) women with menarche age > 15 years [164]. Importantly, the three groups were shown to be identical with regard to HR-HPV prevalence (both HC2 and real-time PCR, TaqMan assay), detection of Pap smear abnormalities (ASCUS, LSIL, HSIL cutoff) and CIN lesions. There was an increasing trend of ever having been pregnant in parallel with older age at menarche ($p = 0.024$). Not unexpectedly, all variables calculated from menarche age (TMI, TMP, TMD) were significantly different in the three groups, being inversely related to the age at menarche. The other significant differences included: 1) number of deliveries ($p = 0.001$), 2) ever had abortions ($p = 0.019$), 3) age at first sexual intercourse ($p = 0.004$), 4) practice of oral sex ($p = 0.001$), and 5) time since the last Pap test ($p = 0.046$) [164].

When the three groups were analysed for the predictors of CIN2+ in univariate analysis, there was no single risk factor in common to all three patient groups. Age below 35 years was most influential (protective) among women who had their menarche at 13-14 years of age, less among those with late menarche (> 15 years), and of no significance in the youngest menarche age group. The HCII+ test was a significant predictor of CIN2+ only in the intermediate menarche age group, as was ever having been pregnant. Interestingly, for HSIL the Pap test failed to correlate with CIN2+ in the older menarche age group, but was a significant determinant in the two others [164].

As compared with predictors of CIN2+, the results were much more unanimous for predictors of HR-HPV. This applies particularly to the young menarche age group and intermediate age group, where practically all predictors were the same, with i) the age of sexual onset, and ii) being a current smoker as the only exceptions. On the other hand, in the older age group, fewer variables were significant predictors of HR-HPV, and the role of casual sexual contacts was a significant risk factor exclusively in this group. The following were the risk factors common to all three groups: 1) age < 35 years, 2) patient category, 3) HSIL in the Pap test, and 4) TMD. All three groups were practically identical in their baseline HPV/Pap status ($p = 0.750$), and no differences could be established between the three groups as to the outcome of their cervical disease (Pap smear abnormality) or HR-HPV infections. These data clearly implicate that the outcome of cervical disease or HR-HPV infections is not related to the menarche age of these women.

In the whole cohort, several variables were highly significant ($p = 0.0001$) predictors of HR-HPV in univariate analysis, but importantly, the age at menarche *per se* was not of any predictive value, irrespective of whether entered as a continuous or categorical variable. On the other hand, TMI, TMP and TMD were all highly significant at the $p = 0.0001$ level. Ever having been pregnant and number of deliveries (abortions, miscarriages) were all protective against HR-HPV [164]. When all highly significant predictors were entered in the multivariate model (including menarche age), only five of these variables proved to be independent significant predictors: 1) age < 35 yrs, 2) patient category, 3) HSIL (all at $p = 0.0001$ level), 4) being a current smoker ($p = 0.014$), and 5) cervical erosion treated ($p = 0.035$). Importantly, menarche age or any of the menarche-derived variables were not independent predictors in the multivariate model.

Finally, multivariate analysis was performed to disclose the independent predictors of high-grade CIN in the whole cohort. Only two of those proved to be significant in the final regression model: i) patient age < 35 years (protective) ($p = 0.0001$), and ii) HC2+ result ($p = 0.014$). Importantly, age at menarche or any of the menarche-derived variables were not included among the independent predictors of high-grade CIN in this multivariate analysis.

Menarche age and the risk of HR-HPV or CIN: conclusions from the NIS cohort

Data from this analysis indicate that menarche age is not associated with increased risk of HR-HPV infections, which are equally prevalent among women with early, intermediate and late menarche [164]. Instead, short intervals between menarche and onset of sexual activity (TMI), first pregnancy (TMP) and first delivery (TMD), are all significant predictors of HR-HPV infection. This impact disappears, however, in multivariate analysis, where the well established risk factors remain as only independent predictors. Similarly, menarche age or any of the three intervals do not predict the development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of cervical lesions or HR-HPV infections in a longitudinal setting over time [164].

Determinants of increased prevalence of HR-HPV infections among older women

Since the early reports on HPV and CIN, epidemiological data from different countries confirmed that the peak prevalence of cervical HPV infections (detected by Pap smear or DNA hybridisation techniques) occurs between 22-24 years of age, with constant decline in parallel with increasing age [22, 165-167]. This was neatly explained by the

early studies (based on Pap smear screening data) implicating a particularly high (8%) annual incidence of HPV infections among 22-year-old women [168, 169].

More recent studies on the natural history of HPV infections [1 ,2] have further refined the dynamics of these viral events in different populations. Accordingly, incident HR-HPV infections are clearly age-dependent, the 3-year cumulative incidence exceeding 50% among women below 20 years of age, following the onset of their sexual activity [65, 170, 171]. On the other hand, clearance of the virus did not show such strict age-dependence [21], but continued at a constant rate among women over 30 years of age [165, 172]. These age-specific incidence and clearance rates used to estimate the age-specific prevalence of HR-HPV infections reproduce the true figures quite closely, except for a small gap in each of the 5-year age groups [67]. This gap between the true- and estimated age-specific prevalence rates is due to the fact that instead of clearance, some of the acquired infections remain persistent. These persistent HR-HPV infections are considered as prerequisite for developing a progressive cervical disease and are currently the subject of intense study for their covariates [66].

Second peak of HPV prevalence among postmenopausal women

During the past few years, this dynamic model of HPV acquisition, clearance and persistence, explaining the linearly declining age-specific prevalence curve [22, 165-167] has been challenged by the data from several population-based studies, reporting a second peak in HPV prevalence among women > 55 years of age [43, 105, 173]. In some studies, a similar peak among older women has been reported for HPV incidence as well [174, 175]. Indeed, some recently published population-based studies report highly contradictory results from different geographic areas. There are populations, where the age-specific prevalence curve is clearly U-shaped, with a second peak among postmenopausal women [43, 105, 173-179]. In other studies, no such U-shaped prevalence curve was established, but the shape was that of a declining linear curve [29, 41, 180-183]. The IARC HPV Prevalence Survey data failed to give one single explanation for these differences, and several key questions still remained unanswered [48, 184].

Analysis of age-specific HR-HPV prevalence in the NIS cohort

In the NIS cohort, we wanted to clarify the reasons for the U-shaped HPV prevalence curve [185], previously reported in this cohort [63]. The whole cohort of 3,187 women was stratified into three age groups according to their different HR-HPV prevalence profile. These three age categories are: i) two youngest age groups (women below 20 years and those between 21 and 25 years; n = 1,103) with the peak HPV prevalence; ii) women between 26 and 55 years (n = 2,004) showing linearly declining HPV prevalence; and iii) women past 55 years (n = 80) with sharply increasing HR-HPV prevalence [63, 67]. To adjust for the differences in age distribution in the three NIS countries, the age-standardised HPV prevalence was calculated for 14 five-year age groups (15-84 years) of the European standard population [185]. Logistic regression modeling with the curve estimation procedure was used to assess the age profile in each three countries, by fitting the logistic regression model with either i) linear, ii) quadratic or iii) cubic terms for those 14 five-year age groups. Curves with a significant ($p < 0.05$) quadratic term were classified as non-linear (U-shaped), those with significant cubic term as non-linear (bi-phasic or S-shaped), to distinguish from those with only a linear age term. All curve fit procedures were controlled by scatter plots, where the fit parameters (= predicted parameters) were plotted against the residuals [185].

Age-specific HR-HPV prevalence in the NIS cohort

The age-standardised prevalence rate (ASPR) of HR-HPV infections was very similar in Russia (18.3/100 women; 95% CI, 16.6-19.9), and Belarus (17.2/100 women; 95% CI, 14.1-20.3), but in Latvia as high as 24.6/100 women (95% CI, 20.60-28.65) [185]. In the whole cohort, HPV prevalence curve was clearly U-shaped, steadily declining from 55.6% among women < 20 years of age, down to 10.1% among those aged 51-55 years, followed by a deep increase among women older than 55 years [185]. In the whole cohort, the F statistic for model fit was significant both in the linear and quadratic equation ($p = 0.0001$), but substantially higher ($R^2 = 0.966$) for the quadratic model (U-shape curve) than ($R^2 = 0.809$) for the linear model. In the curve of Russia, the results mimic those for the whole cohort; $R^2 = 0.806$ for the linear model and $R^2 = 0.968$ for the quadratic model ($p = 0.0001$ for both). In the curve of Belarus, there was not much difference between the linear and quadratic models; $R^2 = 0.952$ and $R^2 = 0.995$, respectively. The age-specific HPV curve of Latvia shows the least obvious linearity and the most accentuated second peak; $R^2 = 0.647$ for linear and $R^2 = 0.915$ for the quadratic model [185].

Epidemiological, clinical and viral determinants of increased HPV prevalence in the older women

The three age categories of women were shown to differ at the $p = 0.0001$ level with regard to the majority of the recorded epidemiological variables [185]. Many of these variables are directly explained by the age difference between the three categories. On the other hand, however, there are some interesting variables that do not show any difference between the three groups; e.g., history of skin and genital warts, time since last Pap smear, previous Pap smear normal, and ever having had cervical erosion [185].

Of the determinants of HR-HPV infection in the three groups, patient category was significant only in the two groups of younger women, but not among the older ones. HSIL Pap predicted HR-HPV only in the two older groups, whereas the CIN3 cutoff was a significant predictor only in women between 25 and 55 years of age. The same holds true with the number of deliveries, which had a protective effect among this age group (a surrogate of regular family life?). A history of previous CIN was significant only among the older women (OR = 5.62; 95% CI, 1.01-31.48) [185].

HPV prevalence was highest among the youngest age group, but not significantly different between the two older ones, irrespective of whether determined by the HC2 or TaqMan assay. The quantitative viral loads for HPV16, 18/45, 31 and 33 were markedly higher among the older women. The most interesting is the curve of HPV16 viral loads, showing the best fit with the cubic model ($R^2 = 0.714$) and resulting in a distinct biphasic S-shaped curve with a sharp second rise among women past 50 years of age.

The distribution of individual HPV types was significantly different among the three age categories [185]. As compared with the youngest age groups, there was a marked shift from multiple-type infections (from 30.7% to 6.3%) to the accumulation of HPV16 (37.5% of all HPV+ cases) and HPV31 (31.3%) among the older women. There was a transition from episomal to mixed and integrated state among the youngest age groups to those above 55 years of age, in whom, all HPV16 positive lesions showed viral integration ($p = 0.009$). Similarly, the viral load of integrated HPV16 was significantly higher (17.5) in the older women, as compared with the two other age categories, with practically identical loads of integrated HPV16 [185].

Finally, we noticed no difference in the clinical course of the cervical disease as determined by the repeated Pap tests [185]. In contrast, the outcome of HR-HPV infections was significantly different among the three groups. As compared with the age group 26-55, in which a sharp decline of HR-HPV prevalence was characteristic, women over 55 years of age showed a i) higher proportion of incident infections, ii) higher rate of viral persistence, and particularly iii) lower rate of HR-HPV clearance ($p = 0.0001$) [185].

Determinants of age-specific prevalence of HR-HPV: conclusions from the NIS cohort

This study sheds new light on most of the open issues related to the shape of the age-specific HPV prevalence curves [43, 105, 173-179], and in particular to the determinants of the second peak observed among women past 55 years of age. Taken together, these data feasibly explain what was suggested by our in vitro studies some years back [186-188]. The rapid acquisition of HR-HPV infections after onset of sexual activity [15, 67] leads to an early peak of both HR-HPV prevalence and viral loads between 20 and 25 years of age. This is followed by a constant clearance [21, 67] and reduced viral loads of the infections between 25 and 55 years of age. In women > 55, a sharp increase in both HPV prevalence and viral loads follows, shown by the U-shaped and S-shaped age-specific curves, respectively [185]. These data implicate that among women who fail to eradicate their HR-HPV infection by menopause, the selection of an integrated viral clone has probably taken place, driving the process towards an aggressively progressing disease. Consequent to this, most of the HR-HPV infections in women older than 55 years were associated with high-grade CIN or invasive carcinoma in the NIS cohort [185].

Are different screening strategies needed for younger and older women?

In the above, the special features of HR-HPV infections encountered in postmenopausal women have been discussed [185]. It sounds feasible to speculate that the optimal screening strategies for these older women might be different from those applied for younger age groups. Indeed, data from several recent trials suggest that screening strategies optimal for women below 30-35 years of age should be different from those used to target older women [59-62, 189-191]. In these trials, HPV testing has been shown to perform better among these older women [192-195], evidently due to the fact that HPV infections among younger women are extremely common, and in most cases resolve without inducing clinical lesions [3-5, 28, 196]. These data further implicate that adjunct HPV testing of these older women might enable extension of the screening interval to three to five years, leading to considerable cost savings in the screening programmes [59-62, 189-192]. Until now, however, these strategies have been tested almost exclusively in well resourced Western countries, where the necessary infrastructure for CC screening exists [59, 60, 62, 189-196], and little data [61] are available on the feasibility of these different screening strategies among non-privileged women in low-resource settings where CC burden is the highest.

Testing optional screening strategies for younger and older women in the LAMS study

In the LAMS study, eight optional screening tools have been compared in a population-based cohort of > 12,000 women in Brazil and Argentina [64, 197-200]. To address the effect of age on screening strategies, we recently compared the performance of conventional Pap smear cytology, liquid-based cytology (LBC), visual inspection with acetic acid (VIA) as well as HPV testing with HC2 assay in two sub-cohorts of these women; those younger than 35 years (n = 5,099), and those older than 35 years (n = 6,997) [201]. The aim was to test the concept as to whether optimal screening results could be obtained by different diagnostic tools among younger and older women, using the biopsy-confirmed CIN2+ as the gold standard with all test performance indicators being corrected for the verification bias.

Data from the LAMS study

Not unexpectedly, the two sub-cohorts analysed in this trial differ in the vast majority of the recorded variables, including the risk factors of HPV, CIN and CC. Although many of these differences are directly related to the different ages of these women, there are numerous indicators of life-style risk of sexual behaviour that are significantly different in these two cohorts [201]. Indeed, the list was very short for those variables that are not significantly different: i) prevalence of HSIL, ii) history of previous CIN or warts, and iii) being a current smoker. The prevalence of HR-HPV infections was almost twice as high (24%) among younger women than that (13.5%) among the older ($p = 0.0001$). The contrary was true with the prevalence of high-grade CIN lesions. Bearing that the performance of the diagnostic test is dependent on the prevalence of the target disease, it can be anticipated that tests detecting HPV and those detecting high-grade CIN will perform differently in these two sub-cohorts [201].

Indeed, this was shown to be the case for all these diagnostic tests when separately analysed in these two sub-cohorts [201]. Starting from conventional Pap smear, we showed that among young women, the HSIL Pap smear is a highly specific test (98.6%) in detecting CIN2+, but suffers from low sensitivity (33.7%). Corrected for the verification bias increases the specificity close to 100% (99.7%), but further reduces the sensitivity. Using the LSIL cutoff slightly increases the sensitivity (SE) (45.3%) at the expense of reduced specificity (SP) and positive predictive value (PPV). Among women above 35 years, the HSIL Pap is an excellent test in detecting CIN2+, with AUC (area under ROC curve) 0.828 (95% CI, 0.779-0.876) and 67.4% SE, 98.2% SP, 87.3% PPV and 94.3% negative predictive value (NPV). Using LSIL cutoff increases SE but compromises SP and PPV, although AUC is practically identical (0.827), and also significantly different from that ($p = 0.684$) among younger women. Correcting for the verification bias makes the AUC values of HSIL and LSIL very similar in both sub-cohorts [201]. It was also shown that the performance indicators of liquid based cytology (LBC) were inferior to those of the conventional Pap smear in all aspects [201]. The results of LBC faithfully reproduce the changes in the indicator values of the Pap test, according to i) SIL cutoff, ii) correction for verification bias as well as iii) the difference between younger and older women. The best balance (AUC = 0.746, 95% CI, 0.560-0.932) is obtained for LSIL cutoff among the older cohort, but the difference between the sub-cohorts is not significant in this or any of the other test comparisons. When corrected for the verification bias, again LBC (HSIL) is an almost 100% specific test both among younger and older women.

Of the two optional screening tools (VIA, HC2), VIA with the regularly used "abnormal" as the cutoff seems to suffer from both low SE and low SP in both sub-cohorts [201], with no difference in AUC values: 0.535 and 0.541 for younger and older women, respectively. Correction for verification bias does not make the difference significant between the two sub-cohorts ($p = 0.824$). Using the "suggesting cancer" as the cutoff makes VIA an almost 100% specific (99.9%) test in detecting CIN2+ in both younger and older women, when corrected for the verification bias. However, because of the rarity of these lesions, particularly test sensitivity drops down to one percent in young women, and remains low (18.5%) even among the 35+ age groups [201]. This increase makes the difference between younger (AUC = 0.505) and older (AUC = 0.589) women significant ($p = 0.0001$), however.

In this comparison, the results on HPV testing as a screening tool are highly interesting. HC2 assay was tested with two thresholds; 1) the manufacturer recommended 1 pg/ml and 2) a higher, 10 pg/ml (RLU/CO) [201]. The results are straightforward; HC2 assay performs significantly better among older women, irrespective of the cutoff, or whether corrected for the verification bias or not. At the best balance, HC2 assay has 85.9% SE, 91.4% SP, 40% PPV and 99% NPV. This (AUC = 0.886) is significantly different from that (AUC = 0.722) for the younger sub-cohort, as are all other comparisons, except the one using the 10 pg/ml cutoff and correction for the verification bias ($p = 0.174$).

When similar figures were calculated for the combined use of conventional Pap and HC2 assay (with 1 pg/ml cutoff) in the two sub-cohorts, changing the Pap smear cutoff from HSIL to LSIL does not significantly affect the test performance. In all situations, this combination works significantly better in older women. The best balance (AUC = 0.919) is obtained when the values for HSIL/HC2 are corrected for the verification bias, and almost as good (AUC = 0.911) by using the LSIL/HC2 combination [201].

Screening strategies should be different for younger and older women: conclusions from the LAMS study

A notwithstanding conclusion from this analysis was that the conventional Pap and HC2, but not LBC and VIA, perform very differently among younger and older women (201). Similar to Western countries, also in this low-resource setting, both HC2 and conventional Pap tests perform significantly better among women older than 35 years. Correcting for the verification bias, however, will make this significance disappear for the Pap test but not for the HC2 assay. This further emphasises the important difference in HC2 performance among younger and older women, because correction for the verification bias is currently regarded as an essential measure to make the results comparable in studies where confirmation by the gold standard is imperfect [202]. The mechanistic explanation certainly includes the important events in HPV biology that emerge among older women. Instead of HPV clearance being the most common event among young women, there seems to be HPV persistence, transition from multiple-type infections to single-type infections, increase of viral load and higher probability for virus integration among the older age groups [185].

Of the single tests, the best performance is obtained for verification bias-corrected HC2 among the older women. If only the PPV is considered, there is no test superior to the conventional Pap test in both younger and older women. Among the latter, the best balance in SE and SP is obtained when HC2 is combined with the Pap test, whereas in younger women, such a combination does not give any added value to HC2 as the stand-alone test (AUC = 0.728 and 0.721, respectively), and adds very little to the conventional Pap test (AUC = 0.662 for HSIL and AUC = 0.684 for LSIL). The choice of an optimal screening test for younger and older women depends on whether the highest PPV (Pap test) or the best SE/SP balance (HC2) is used as the selection criteria [201].

Conclusions and future prospects

The NIS cohort and LAMS study have significantly contributed to solving several of the open issues in the natural history of HR-HPV infections, including the risk factors, covariates necessary in cervical carcinogenesis as well as in sorting out the optional screening strategies in low-resource settings and for women in different age groups. Many of these issues have been discussed in this communication. However, still several important issues remain to be elucidated before the complex natural history of HPV infections and their role in cervical carcinogenesis can be understood [203].

Accordingly, in light of the existing literature, the role of OC as a risk factor of CC still remains enigmatic. Data obtained from the NIS cohort [124], however, suggests that i) the sexual behaviour is different among OC users, non-OC users, and non-users of contraception; ii) these different risk factors predispose women to HR-HPV, development of high-grade CIN (HSIL), and also influence the outcome of their cervical disease/HR-HPV infection, which is similar irrespective of their OC status, and iii) the use of OC is not an independent risk factor for any of these intermediate endpoint markers in cervical carcinogenesis. Failure to record the epidemiological data on the sexual behaviour and gynaecological and obstetric history inevitably leads to erroneous conclusions on the role of OC as an independent risk factor of cervical cancer and its precursors [124]. In all future studies, the strong confounding effects from these lifestyle behavioural factors must be taken into account, while interpreting the data on OCs as potential risk factors of CC.

Similarly, the published data on the role of smoking as a risk factor of CC remains controversial. This is particularly due to the fact that too few population-based studies are available, where the evident strong confounding effects from the HR-HPV infections would have been adequately controlled. Our results from the NIS Cohort [146] failed to provide any evidence that cigarette smoking is an independent risk factor of CIN2+. Smoking status seems to be strongly confounded by sexual habits, and few predictors of HR-HPV and CIN2+ were common to never smokers, past- or current smokers. There was no indication whatsoever that increase in the risk of CC is mediated by increased progression of the disease among smokers. Instead, the increased risk (if any) of CC among smokers seems to be attributed to the increased acquisition of HR-HPV infections, of which smoking status was an independent predictor in the multivariate model (146). This fact must be adequately controlled in all future studies analysing the role of smoking as a covariate in cervical carcinogenesis.

The same seems to apply to drug addiction as a risk factor of CC as well. The analysis of the LAMS data was the first to address the importance of drug abuse as an independent risk factor of both HR-HPV infections and CIN2+ lesions, using a matched case-control setting [160]. These analyses confirm that drug abuse itself is not a risk factor of i) contracting oncogenic HPV infection or ii) developing CIN2+. In simple terms, drug abuse seems to be closely associated with several of the key indicators of risky sexual behaviour, which predisposes women to oncogenic HPV infections, and thus indirectly contribute to the development of CIN2+ lesions. Clearly, more studies are needed on this subject, based on large enough sample sizes to enable sub-group analysis of individual drugs, which was not possible in our study [160].

As to the role of reproductive factors as possible covariates, data from the NIS cohort clearly implicate that menarche age is not associated with increased risk of HR-HPV infection [164]. However, short intervals between menarche and onset of sexual activity, first pregnancy and first delivery, were all significant predictors of HR-HPV infection in univariate but not in multivariate analysis. Similarly, age at menarche or any of the three intervals (TMI, TMP, TMD) did not predict the development of high-grade CIN, feasibly explained by the fact that menarche age does not have any effect on the outcome of cervical lesions or HR-HPV infections during a prospective follow-up of this cohort [164]. This topic still merits further studies, however, to elucidate e.g., whether these results elaborated collectively for HR-HPV types also hold for individual HPV genotypes.

Another special group are postmenopausal women, now shown in several studies to have a second peak of HR-HPV prevalence in many populations [43, 105, 173-179]. In the NIS cohort, rapid acquisition of HR-HPV infections after onset of sexual activity [15, 67] leads to an early peak of both HR-HPV prevalence and viral loads between 20 and 25 years of age. This is followed by a constant clearance [21, 67] and reduced viral loads of the infections between 25 and 55 years of age. In women > 55, a sharp increase in both HPV prevalence and viral loads follows, shown by the U-shaped and S-shaped age-specific curves, respectively [185]. These data implicate that in women who fail to eradicate their HR-HPV infection by menopause, there is a transition from multiple infections to single-type infections, most probably accompanied by selection of an integrated viral clone, driving the process towards an aggressively progressing disease [185]. This special group of women is currently under intense study in several population-based studies.

These peculiarities in the behaviour of HR-HPV infections among older women finally leads us to consider the issues, whether different screening strategies would be appropriate for younger and older women [201]. Consonant with other recent reports [59-62, 189-192], data from the LAMS study showed that conventional Pap and HC2, but not LBC and VIA, perform significantly differently among younger and older women [201]. However, the choice of an optimal screening test for younger and older women depends on whether the highest PPV (Pap test) or the best SE/SP balance (HC2) is used as the selection criteria [201]. It is generally agreed that an optimal screening test is the one with the highest PPV (204). A high PPV suggests that a reasonably high proportion of the programme costs are being spent for detection of true disease, while low PPV indicates that a high proportion of costs are being wasted for the evaluation of the false positives using other diagnostic tests. At the end of the day, it is most likely the cost-effectiveness that is the decisive factor of which screening test will be selected, at least in the low-resource setting. Needless to re-iterate that screening for cervical cancer precursors will be mandatory until the foreseeable future, even in this emerging era of prophylactic HPV vaccination.

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