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Mechanisms and predictors of high-risk human papillomavirus (HPV) clearance in the uterine cervix

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Summary

Cervical cancer (CC) and its precursor lesions (CIN) are unique in that we can study the natural history of one disease at two different levels; i) by assessing the clinical lesions, and ii) by analysing the viral events of human papillomavirus (HPV) infections, their prime etiological agent. In this review, we are interested in mechanisms and predictors of clearance of oncogenic HPV infections in the uterine cervix.

The outcome (natural history) of CIN has been well established by a large number of prospective cohort studies covering over 25,000 patients, and the figures for regression, persistence and progression are well established. The outcome of HPV infections is far more complex with at least six distinct patterns being demonstrated in long-term cohort studies. There is little doubt that the mechanistic explanation for HPV clearance is by specific immunological reactions, where competent humoral and cell-mediated immune mediators are needed. To understand this process in detail still necessitates a substantial amount of clinical and laboratory research, however.

In general, HPV outcomes follow the pattern where a dynamic balance exists between incident infections and virus clearance. Following a rapid accumulation of incident infections after onset of sexual activity (women < 20 years of age), there is a transition of this balance in favour of virus clearance soon after age 25. This explains the constantly declining age-specific prevalence of HPV infections until menopause. Failure to eradicate the virus at postmenopause is not uncommon, however, explaining the deep second peak in HPV prevalence now reported in many different populations.

The importance of HPV clearance/non clearance (= persistence) has been recognised recently, and the number of studies addressing these issues has increased substantially during the past few years. The data are now rather unanimous concerning the times and rates (usually expressed per 1,000 women/months at risk, WMR) of HPV clearance. On the other hand, data are still incomplete and in part inconsistent as to the cofactors that regulate these events. A wide variety of variables have been explored as potential co-determinants and/or predictors of HPV clearance, as reviewed in this communication. Until now, all efforts attempting to identify suitable biomarkers as such predictors, have been disappointing, but fortunately, this is a largely unexplored area as yet.

Similarly, data on the two extremes of life, i.e., early infancy and postmenopause, are still far too fragmentary to enable creating a comprehensive view, how these viral infections behave in early life, and what makes many women incapable of clearing their virus at postmenopause. Both issues are of utmost importance and have widespread clinical implications; we need to know how and why some infants and children contract HR-HPV infections well before the onset of their sexual activity, to be able to select the proper targets for prophylactic HPV vaccination. Similarly, we need to know why some women over 55 years of age are likely to remain HR-HPV carriers, while the vast majority successfully clears their infection well before the menopausal age. Early detection of cervical cancer precursors among these elderly HR-HPV positive women past the usual age of organised screening remains a major challenge also in the future.

Key words: Human papillomavirus (HPV); Clearance; Outcome; CIN; Cervical cancer; Natural history; Predictors; Biomarkers.

Introduction

The data from carefully controlled, long-term prospective follow-up studies suggest that the natural history of clinical human papillomavirus (HPV) infections of the uterine cervix is basically identical to that of cervical cancer precursor (CIN = cervical intraepithelial neoplasia) lesions, with a) progression, b) persistence, and c) regression as the three main outcome measures [1-3]. 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 HPV type, viral load, acquisition of new (incident) infections as well as clearance of the virus and 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 the 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, both reporting conflicting findings [16-21].

Prompted by these inconsistent data, we recently carried out a systematic approach to study these dynamic events in the natural course of high-risk (HR)-HPV infections in a cohort study, where almost 3,200 women were tested for optional screening tools of cervical cancer in three New Independent States (NIS) of the former Soviet Union (Russia,

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Belarus, Latvia), and prospectively followed-up to study the natural history of cervical HPV infections (the NIS Cohort study, to be repeatedly cited in this text) [22, 23]. We observed that in baseline HR-HPV negative women, the acquisition time for HR-HPV is three months shorter than that of an abnormal Pap test [15]. Young age and being an STD patient were independent predictors of incident HR HPV infections. In another sub-cohort of baseline HR-HPV positive women, the clearance of HR-HPV DNA and abnormal Pap test showed a close temporal relationship, the former preceding the latter by an interval of one to two months [21]. Finally, in a study where both incident infections and HPV clearance were modelled together, we described that accumulation of incident HR HPV infections is significantly age-related, whereas virus clearance is relatively constant among women between 15 and 60 years of age [24]. Using these distinct age-specific incidence- and clearance rates, we could very closely calculate the age-specific prevalence of HR-HPV infections in our NIS cohort.

Since identifying the women at low risk for development of high-grade CIN and CC is essential, elucidating the factors involved in clearance of HR-HPV infections is of key importance. This review is specially focused on the mechanisms and predictors of clearance of HR-HPV infections in the uterine cervix. Because of the ambiguous usage of the term clearance, the topic had to be restricted by strictly adhering to those "spontaneous" events through which cervical HR-HPV infections are cleared (instead of remaining persistent).

Thus, excluded from this discussion are the following clearance-related topics: 1) clearance following the systemic or topical treatment of HPV infections with chemotherapeutic agents (e.g. interferons, imiquimod, podophyllin, etc.) [25, 26], 2) clearance by HPV vaccination, and 3) papillomavirus clearance demonstrated in animal models that are exhaustively discussed elsewhere [3-5]. In addition, mechanisms of HPV clearance following local ablative or destructive treatment of cervical lesions are discussed only as far as pertinent to the role of HR-HPV in disease eradication or recurrence [27]. Before entering in the discussion about the issues related to HPV clearance, however, the natural history of cervical cancer precursors and their causative HPV infections are addressed in brief.

Natural history of cervical cancer precursors

The natural history of cervical cancer precursors has been firmly established in a large number of prospective cohort studies conducted well before "the HPV era", starting from the early 1960s [2, 3, 7]. This literature was exhaustively reviewed by Östör in 1993, who made a meta-analysis of the available data based on follow-up of over 25,000 women with different grades of CIN [1]. According to these figures, disease progression is directly- and regression inversely related to the grade of CIN lesion at baseline (Table 1). In this table, the figures for progression to invasive cancer are clearly underestimates because they are not based on reliable follow-up data, which are impossible to obtain if ethical rules are strictly followed. To make the long story short, there is no doubt, however, that low-grade CIN lesions possess a remarkable potential for spontaneous regression, if left untreated [1-4, 7]. At the other end, high-grade CINs are true cancer precursors with substantial tendency for progression, but yet a small minority seems to be capable of resolving as well (Table 1).

Table 1. — *Natural history of CIN and cervical HPV lesions.*

Lesion grade	Regression	Persistence	Progression to CIS	Progression to invasive CC
CIN I	57%	32%	11%	1%
CIN II	43%	35%	22%	5%
CIN III	32%	< 56%	–	> 12%
HPV-NCIN	79.9%	14.6%	5.2%	0%
HPV-CIN I	65.1%	20.8%	14.2%	0%
HPV-CIN II	58.6%	18.6%	21.4%	0%
HPV-CIN III	11.6%	9.3%	79.1%*	0.5%[1]

Table compiled from the data reviewed by Östör [1], and from the data of Kuopio Prospective Follow-up study (1981-1998), discussed in Syrjänen *et al.*, 2000 [2].
*Progression based on colposcopy (lesion severity and extent) and histology (progress from severe dysplasia to CIS in two subsequent biopsies); CIS, carcinoma in situ; HPV-NCIN, HPV lesion without concomitant CIN.
[1]One lesion progressed to invasion in less than 3 years (Ref. ref. 2,7).

Natural history of cervical HPV lesions

There is little room for doubt that oncogenic HPV types are the single most important causal agents of CIN and CC [2, 3, 5, 6]. Since the recognition of this link in the late 1970s, a growing number of prospective cohort studies were designed to assess the natural history of cervical HPV lesions (discussed in 4). By the late 1980s, it became evident that the natural history of HPV infections in the uterine cervix is more complex than merely regression, persistence or progression, which are the well established outcomes of CIN lesions. Based on long-term (> 10 years) monitoring of over 500 women, we could establish at least six different outcome patterns for cervical HPV infections: 1) early regression, 2) persistence, 3) fluctuation, 4) late regression, 5) progression, and 6) recurrence [2, 7].

When fitted in a model with three major outcomes (regression, persistence and progression), we were amazed by the similarity of the natural history of cervical HPV lesions in our cohort and that of CIN lesions reported in the world literature (Table 1). As shown by these data, these figures are practically identical particularly for the low-grade lesions. This unified natural history is not unexpected, however, taken that HPV infection is the etiological agent of CIN, and any result divergent from this would, indeed, violate this concept [1, 2, 7].

Among all human malignancies, cervical cancer and its precursors are unique in that we have the means to monitor the natural history of the disease at two different levels: 1) disease outcome by means of colposcopy, Pap smear and biopsy, and 2) the outcome of viral infection, using sensitive molecular techniques. As it now seems, both are needed for the overall adequate management of these patients. In the subsequent section, we will discuss the current level of understanding, why HR-HPV infections in the vast majority of women will undergo spontaneous clearance, and why some (a small minority) fail to eradicate the virus and consequently are exposed to a significantly increased risk of progressive cervical disease [2-10, 12].

Morphological evidence for regression of HPV lesions

According to current concepts, there is little doubt that HPV infections (like all other viruses) are eradicated by the host immune mechanisms [2-5, 28, 29]. Both systemic humoral and cell-mediated immune response are needed, exhaustive discussion about the details falling clearly outside the scope of this communication, however [28, 29]. In addition to the systemic immunity mediated by circulating antibodies and activated immunocompetent cells, local immune reactions at the lesion site are likely to play an important role in host resistance against many (if not all) HPV infections [29, 30]. At the tissue level, such a local immune reaction mounted against virus-infected cells is manifested as detectable infiltrations of small lymphocytes, among which, cytotoxic (CD8+) T cells are most numerous in cases where cytotoxic response is the dominant mechanism of virus eradication [29, 30].

Introduction of the newly invented monoclonal antibody technology to serve the large-scale production of commercial antibodies in the early 1980s made it possible to dissect these local inflammatory infiltrates at the level of individual cell types, a process known as immunophenotyping [30]. Starting with cutaneous HPV lesions (skin warts), such analyses were soon extended to cervical biopsies as well. Indeed, our group was the first to make a systematic analysis of a large series of cervical HPV lesions using immunophenotyping with monoclonal antibodies in the early 1980s [31-36]. In these studies, we were primarily interested in assessing whether any changes in the proportions of the subsets of these local immunocompetent cells: e.g., the ratio of CD4+/CD8+ (Th/Ts), HNK-1 (Leu-7)-positive NK (= natural killer) cells, or OKT6 + Langerhans cells, would bear any correlations with the outcome of cervical HPV lesions in our prospective follow-up cohort.

Albeit constantly present within the inflammatory cell infiltrates subjacent to HPV lesions, these cell types and their enumeration unfortunately provided little help in predicting the outcome of cervical HPV infections [31-36]. One of the problems with these early studies was the failure to assess whether such an inflammatory cell reaction is specific to the antigenic determinants of HPV-infected cells or merely reflects a non-specific inflammatory reaction to developing neoplastic process in the epithelium [29, 30]. Such a demonstration had to await the late 1990s, when Evans *et al.* (1997) showed that cytotoxic T-cells in these infiltrates were, indeed, HPV-specific [37]. More recently, Monnier-Benoit *et al.*, showed that CD4+ cells predominated in regressing CIN1 which had the highest CD4+/CD8+ ratio, as compared with progressive CIN1, CIN3 and invasive carcinoma [38], suggesting an active role in the natural history of HR-HPV-associated lesions.

Even so, such immunophenotyping of the stromal (subepithelial) inflammatory cells is of relatively limited value in explaining the virus-specific immunity in HPV lesions. To explore these, specific functional studies of the effector cells are necessary, as discussed in more detail elsewhere [29, 30]. Interesting recent data on these lines implicate that HPV-specific CD4+ T-cell response might be important in HPV clearance [39]. These authors demonstrated in ex vivo experiments significant differences in HPV16 immunity during progressive CIN, concluding that the HPV-specific CD4+ T-cell response might be an important candidate in immunotherapy designed to target precancerous lesions. In another recent study, interleukin (IL) 10 polymorphism (G allele) at position -1082 was associated with clearance of HPV infections in a series of 166 women [40].

Taken together, as suggested several years ago [31-36], local HPV-specific immune response seems to play an important role in eradication of the virus in cervical epithelium. Even if accepted that both humoral and cellular immunity is needed for effective clearance of HPV [29, 30, 41-46], the basic question still remains unanswered, why some people fail to eradicate the virus, while the vast majority does get rid of HPV within a relatively short time after initial exposure. Answers to this basic question have been searched for in a large number of recent studies, where a wide variety of co-factors have been explored as potential predictors of HPV clearance in different clinical settings.

Outcome of cervical HPV infections

Adhering to strictly virological terms, HPV infections can be classified as i) latent, ii) subclinical, and iii) clinical infections [2, 5, 9, 10, 12, 29]. Unfortunately, the terms latent and subclinical have been used interchangingly in the previous literature, which has caused some confusion, particularly when very little is known about the mechanisms of HPV latency [2, 5, 29]. Not unlike other viruses, HPV infections have three possible outcomes in the infected cells: i) incident (new) infection, ii) virus persistence, and iii) clearance of the infection. The dynamics of these viral outcomes has been explored in more detail only relatively recently [12-21].

Clearance of cervical HPV infections

As discussed above, the six different outcome patterns of cervical HPV lesions (clinical disease) include two distinct patterns of regression, known as i) early regression, and ii) late regression, defined using the criteria discussed elsewhere [2, 7]. Accordingly, these two regression patterns denote a rapid (early) or delayed (late) resolution of biopsy-confirmed HPV lesions on colposcopy and biopsy as well as disappearance of cytological abnormalities in the Pap smear. Whether the same happens to HPV DNA and in which sequence (i.e., before or after resolution of the clinical disease) has remained an enigma until recently, when this issue has been addressed by using well standardized and highly sensitive techniques for HPV detection [16-21].

Clearance times and clearance rates

Calculations for clearance times and monthly clearance rates for HPV have been presented only recently [12-14, 16, 20]. Franco *et al.* [12] showed that the monthly clearance rate was higher for non-oncogenic types (12.2/1,000 WMR) than for oncogenic HPV infections (9.5/1,000 WMR). These monthly clearance rates are close to those reported by us [21]. The same is true with the mean infection durations (13.5 months), which are close to the mean clearance times for HR-HPV in our series (12.6 and 10.8 months) [21]. These two studies are also consonant in that the woman's age did not affect the mean duration of oncogenic HPV infections (13-14 months) [12]. In the study of Guiliano *et al.* [13] the median time to clearance of HPV infection was significantly longer for oncogenic types (9.8 months) than for non-oncogenic HPV (4.3 months). In our study, we did not analyse the non-oncogenic HPV types. Sellors *et al.* [14] showed that of the 54 previously HPV-positive women, 51.9% (28/54) had cleared the infection in one year. For comparison to our data, 68.6% of women in Group 1 (women who cleared both Pap and HPV) and 34.4% in Group 2 (women who cleared either Pap or HPV) had experienced a clearance of HPV at 14 months of mean follow-up [21]. Zielinski et al. [20] stated that the median clearance time for abnormal cytology in HPV-positive women was 1.9 years (22.8 mo), and that of HR-HPV DNA was 1.7 years (20.4 mo) [20]. Compared with our data, these median clearance times for both HPV and Pap are significantly longer, suggesting a higher rate of acquisition of new (incident) infections in their series [20]. In another recent study, the majority of type-specific HPV infections cleared within two years, but there were many women who were either re-infected with a new HPV genotype or experienced reactivation of their initial infection [47].

Temporal relationships to clearance of cervical lesions

There are two contradictory opinions concerning the temporal relationships between the clearance of HR-HPV DNA and the respective Pap smear abnormality [16, 17, 20]. While Nobbenhuis *et al.* [16] and Zielinski *et al.* [20] conclude that the clearance of HPV DNA precedes the clearance of an abnormal Pap smear by an average of three months [16] or 0.3 years [27], Schiffman *et al.* [17] provide evidence that HPV DNA remains detectable (5-6 months) longer than an abnormal Pap smear. In another recent study, Winer *et al.* [48] reported the median time to clearance of cervical and vaginal SILs as 5.5 and 4.7 months, respectively.

Reasons for these discrepant observations have been extensively discussed in a recent report [17]. On the basis of the known biological behaviour of HPV infections in the cervix [2, 3, 12], it is not easy to find a feasible explanation for such a long gap between the two clearance events in either direction, i.e., a three to four months interval from disappearance of HPV DNA to the disappearance of the abnormal Pap test [16, 20], or a five to six months interval from disappearance of the Pap to clearance of the viral DNA [17], suggesting that the clearance of HPV DNA and an abnormal Pap test must be temporally more closely bound. Indeed, there was a fair to good correlation between clearance of cervical cytology and HPV DNA tests reported in a recent study [49].

Our recently reported data [21] are more consistent with that of the European authors [16, 20]. Concerning the temporal relationship between the clearance of HPV DNA and abnormal Pap smears [16, 17, 20], our results suggest that the HPV DNA is cleared first, followed by the clearance of an abnormal Pap test, but the interval is not more than one- to two months [21]. Thus, clearance of HR-HPV and an abnormal Pap test shows a close temporal relationship, the former preceding the latter, however, with an interval of one to two months maximum. The clearance rates for both are very similar (in the order of 15/1,000 WMR), and we failed to establish any statistical differences in the cumulative clearance of HPV and an abnormal Pap in life-table modelling [21].

Dynamic balance between HPV clearance and acquisition of new infections

Cumulative clearance of HPV DNA and Pap smear abnormalities are clearly time-dependent phenomena, counterbalanced by the rates of acquisition of new HPV infections and Pap smear abnormalities. Clarifying both the acquisition and clearance rates in different age groups should help estimating the rates of prevalent HPV infections and abnormal Pap smears in sexually active age groups [21].

We recently conducted a detailed analysis on this dynamic balance between acquisition and clearance of HR-HPV infections in different age groups of women included in our NIS cohort [24]. The incidence rates of HR-HPV were significantly age-dependent ($p = 0.0001$), whereas clearance rates remained constant across the nine age groups (< 20

to > 65 years) included in the analysis (p = 0.920). The incidence rates (3.04% and 2.65%) exceeded the clearance rates in the two youngest age groups only, and remained lower (0%-0.84%) in all other age groups. The cumulative rate of incident HR-HPV infections (1.0%) was significantly lower than the overall clearance rate (1.9%) (p = 0.001). In the life-table analysis, incident HR-HPV infections between the nine age groups were significantly different (p = 0.0001), while cumulative HR-HPV clearance was identical in all age groups (p = 0.822). These results implicate that accumulation of incident HR-HPV infections is significantly age-related, whereas virus clearance remains constant between 15 and 65 years of age [24]. These distinct age-specific incidence and clearance rates explain the differences in age-specific prevalence of HR HPV infections in the study population [22].

Table 2. — *Potential co-factors and their influence on HR-HPV clearance.*

Co-factor	Enhance HPV clearance	Prolong/Halt HPV clearance	Data inconsistent	Essential data missing
Humoral immune responses	YES			YES
Cellular immune responses	YES			YES
Age (early infancy)	?	?		YES
Age (sexually active)	Clearance rate is not age-dependent		Cumulative clearance increases until age 55	YES
Age (postmenopause)		in some women		YES
Parity			YES	YES
Pregnancy		YES?	YES	YES
Mode of contraception:				
OC	No effect	No effect		YES
Condom use	YES?			YES
No. of sexual partners	Single: YES?	Multiple: YES?	YES	YES
Immunosuppression		YES		
Current smoking		YES	YES	YES
Diet and micronutrients	YES?			YES
Viral load	No effect?	High: YES?	YES	YES
HPV types	LR-HPV: YES	some HR-HPV: YES	YES	YES
Virus integration	No effect?	No effect?		YES
HPV type variants	?	No effect?		YES
DNA ploidy	Normal: YES	Abnormal: YES		YES
Biomarkers	None shown	None shown		YES
Treatment	YES			
Biomarkers after treatment	None	None		YES

Epidemiological factors predicting HPV clearance

During the past few years, an increasing number of studies have analysed potential predictors of HPV clearance in the cervix. Elaborating such predictors would have important clinical implications in the management of these patients, e.g., avoiding unnecessary treatments of women who will eventually clear their HR-HPV infections. These interesting new data are discussed in the following and summarised in Table 2.

Age

Since the very beginning, it was recognised that HPV infections are extremely common among young sexually active women. The vast majority of these infections are only transient in nature, however, and undergo spontaneous clearance in a relatively short time, i.e., within months to two years [2-5, 12-14, 16, 20, 21, 47-50]. With increasing age, incident HPV infections decrease whereas persistent infections increase, being consistent with the accumulation of high-grade lesions among women older than 35 years. Age itself is not an independent predictor of HPV clearance, however [21]. In contrast to acquisition, which is clearly an age-dependent phenomenon [15], clearance seems to be age-independent, proceeding with a relatively constant rate across all age groups [20, 24]. In fact, monthly clearance rates exceed the monthly acquisition rates already in women past 25 years of age, which neatly explains the declining age-specific HPV prevalence rates in the female population, as previously discussed [24].

At the other end of the age spectrum, however, a second peak of HPV prevalence has been reported in several recent population-based studies (reviewed in 51). Such a deep increase among postmenopausal women leads to an age-standardized prevalence curve that has an almost perfect match with U-shaped curve, e.g., $R^2 = 0.966$ in our study [51]. In this detailed assessment of our NIS cohort, we identified several factors explaining this increased HR-HPV prevalence among older women. These include: i) cohort effect, ii) higher viral loads for HR-HPV types with the cubic model curve ($R^2 = 0.714$) for HPV16, iii) distinct shift (p = 0.0001) from multiple-type infections to single HR-HPV types, iv) transition from episomal to integrated HPV16 (p = 0.009), v) higher load of integrated HPV16 (p = 0.009), and, vi) higher proportion of incident infections, higher rate of viral persistence, and lower rate of HR-HPV clearance. These data suggest that in women who fail to eradicate their HR-HPV infection until menopause, selection of an integrated viral clone has taken place, driving the process towards progressing disease. Consequent to this, most of the HR-HPV infections in women > 55 years were associated with high-grade CIN or invasive CC [51].

Parity

In several large studies, parity has been linked with an increased risk for CC and its precursor lesions [3, 4]. Less data are available on the effect of parity on HPV clearance, however. Molano *et al.* [52] recently studied 227 cytologically normal, HPV+ positive women included in a population-based cohort of 1,995 women aged 13-85 years studied in Colombia. HPV 16 had a significantly lower clearance rate than infections with low-risk (LR)-HPV types (RR =

0.47, 95% CI, 0.32-0.72). Infections with single and multiple HPV types had similar clearance rates. There was no evidence of a dose-response relation between clearance and viral load. Interestingly, they observed a slower clearance in parous women (RR = 0.64, 95% CI, 0.47-0.89) and faster clearance in women who never used oral contraceptives [52]. This is consonant with our NIS cohort data implicating that parous women have a longer clearance time (20.3 mo) of HR-HPV (HCII assay data) as compared with nulliparous women (17.3 mo) (p = 0.021) (unpublished data from the NIS Cohort study). This difference disappears, however, if the type-specific clearance times are calculated using TaqMan assay-based data on specific HR-HPV types [53]. Similarly, there was no direct trend between increased parity and time of HR-HPV clearance, assessed for HR-HPV types collectively (HCII) or type-specific for the most common HR-HPV types (TaqMan).

Pregnancy

Apart from parity, pregnancy itself has been implicated as increasing the risk of HPV infections [3, 4, 6, 18, 29]. The effects of pregnancy were specifically assessed by Nobbenhuis *et al.* [18], who followed 353 women referred with abnormal cervical cytology in a non-intervention cohort study. In 91 pregnant women, the authors compared HR-HPV rates in the subsequent trimesters and postpartum with those in 262 non-pregnant women. The major aims were to test, 1) do HR-HPV rates change during pregnancy? and 2) is there any difference between HR-HPV clearance in pregnant and non-pregnant women? Interestingly, pregnant women showed a trend toward increased HR-HPV clearance during the third trimester and postpartum as compared to non-pregnant women (HR = 3.3, 95% CI, 0.8-13.7) and (HR = 4.6, 95% CI, 1.6-12.8), respectively. These results suggest a lowered immune-response against HPV during the first two trimesters of pregnancy with a catch-up postpartum [18].

Mode of contraception

Another well established risk factor for HPV infections is the mode of contraception, non-use of condom and long-term usage of oral contraception (OC) being implicated as increasing the risk [3, 4, 6]. In our NIS cohort, we stratified the women into three groups according to their contraception modes: i) non-users of contraception, ii) non-OC users, and iii) OC users, and analysed them for predictors of three outcome measures: a) exposure to HR-HPV; b) progression to high-grade CIN (CIN2/3), and c) persistence/clearance of HR-HPV and cytological abnormalities during a prospective follow-up [54]. We found that the outcomes of cervical disease (Pap abnormality) and HR-HPV infection were unrelated to contraception. In a multivariate regression model, mode of contraception was of no predictive value for either HR-HPV or high-grade CIN. We concluded that the use of OC is not an independent risk factor for any of these intermediate endpoint markers of cervical carcinogenesis. Importantly, this includes the clearance and acquisition of HR-HPV infections [54].

The effects of condom use on clearance of HPV were evaluated in another recent study [55], where women with CIN and their male sexual partners were randomized for condom use (condom group n = 72 and non-condom group n = 76). Outcomes of interest were clinical regression of CIN at colposcopy and clearance of HPV. Women in the condom group showed two-year cumulative HPV clearance of 23% vs 4% in the non-users of condoms (p = 0.02). Although lower regression rates were found if women were HPV-positive and had CIN2 lesions at baseline, effects of condom use were found both in women with CIN1 and in women with CIN2 lesions. The authors concluded that condom use promotes regression of CIN lesions and clearance of HPV [55, 56, 63]. In our NIS cohort, we have not yet analysed the effect of condom usage on the outcomes of HR-HPV infections [54].

Number of sexual partners

Early onset of sexual activity and a large number of life-time and/or recent sexual partners is an accepted risk factor for incident HPV infections [3, 4, 6, 29]. Surprisingly few studies have addressed the role of partner number as a determinant of HPV clearance, however. Shew *et al.,* tested 49 HPV-positive adolescents by repeated HPV assays and sexual behaviour diaries [57]. The main observation was that not having multiple sexual partners during HPV infection was associated with early HPV clearance (HR = 5.52, 95% CI, 3.28-9.30). For curiosity, we checked our NIS cohort [22, 23] databank to see whether the number of sexual partners has any effect on HPV clearance time (type-specific and HR-HPV collectively) or clearance frequency in this cohort. We failed to ascribe any affect on either clearance rate or clearance time to the number of recent (within 24 months) sexual partners, however (unpublished). This is clearly one of the subjects that deserves further study.

Immunosuppression

Immunosuppression for any reason is another established risk factor for HPV infections, high-grade CIN and CC [3, 4, 29]. Several recent studies have also specifically addressed the issues related to HPV clearance in such patients, most notably in HIV-infected women [58-60]. As part of a longitudinal study in Baltimore, 268 women (184 were HIV+), were analysed for HPV positivity and time to HPV clearance according to their HIV serostatus and CD4+ cell count [58]. As compared with HIV-negative women, the relative incidence of HPV clearance was only 0.29 and 0.10 among

HIV+ women who had CD4+ > 200 and < 200 cells/μl, respectively ($p < 0.001$). These data are very similar to the observations reported by us in an Italian cohort of HIV- and HIV+ women [59]. We noticed that the clearance rate was significantly less frequent in HIV-positive than in HIV-negative women, 69.2% vs 22.8%, respectively (OR = 0.33, 95% CI, 0.163-0.670), whereas the appearance of new HPV infections was significantly more frequent in the former (OR = 8.8,95% CI, 1.19-64.61). In another recent study, HR-HPV types had low estimated clearance rates relative to LR-HPV types, but HR types were not substantially modified by HIV serostatus [60]. Taken together, these data implicate that HIV-infected women, even on highly active antiretroviral therapy, demonstrate a more aggressive clinical course of cervical HPV infections, and more frequently fail to eradicate the disease than HIV-negative women [58-60].

Role of smoking

Smoking has been listed among the risk factors predisposing women to HPV infections, CIN and also CC [3, 4, 23]. Until now, the effect of smoking on HPV clearance has been analysed in only a few studies [19, 62]. In a prospective cohort of 346 women, the authors examined the probability of clearing and duration of HR-HPV infections by smoking status. Irrespective of whether HCII or PCR was used, smokers maintained an HPV infection significantly longer (median duration of 8.5 months vs 10.7 months), and had a lower probability of clearing HR-HPV infection as compared with women who never smoked [19]. Furthermore, smoking duration was significantly associated with HPV clearance, and a dose response was observed. Interestingly, older age (> 13 years) at smoking initiation was significantly associated with a reduced probability of clearing HR-HPV infection. This was the first study to demonstrate that smoking increases the duration of oncogenic HPV infections and decreases the probability of their clearing [19]. This observation was indirectly confirmed in our NIS cohort, where of all the factors tested, being a current smoker proved to be the only independent predictor of HR-HPV persistence in multivariate analysis [61]. Smoking did not have any independent predictive value on virus clearance, however, and the time to HR-HPV clearance was similar among smokers and non-smokers [15, 21].

In another recent study, never smoking was associated with reduced clearance of HR-HPV in HIV-seronegative women (HR = 0.51, 95% CI, 0.30-0.88) but not in HIV-seropositive women (HR = 0.96, 95% CI, 0.65-1.42) [62]. Current smoking was not associated with clearance of any type-specific HPV in HIV-seropositive or HIV-seronegative women, and HPV clearance did not appear to vary by amount or duration of smoking. The authors stated that smoking does not modify overall clearance but was associated with lower HR-HPV clearance among HIV-seronegative women [62]. It must be concluded that the data reported on the role of smoking as a predictor of HPV clearance are still incomplete and in part controversial, necessitating more detailed studies on this subject.

Effect of diet and micronutrients

There is a growing body of information concerning the influence of nutrition on incident HPV infections, development of CIN and CC [3, 4, 29]. Until recently, the epidemiological data on the importance of nutrients in the development of HPV infections have been inconsistent, and only few studies have specifically focused on nutrients and HPV clearance [63-65].

In a carefully conducted trial, female university students (n = 621) in Montreal were followed-up for 24 months at 6-month intervals by repeated HPV DNA testing and typing [63]. Crude and adjusted hazard ratios of clearing were calculated separately for type-specific HR- (n = 222) or LR- (n = 105) HPV infections. The authors noticed that daily consumption of vegetables seemed to increase the rate of HPV clearance, independent of the virus type. Interestingly, regular condom use was associated with an increased clearance of LR-HPV types only [63].

In another study assessing micronutrients and HPV clearance, women with higher folate status were significantly more likely to become test-negative during the study (OR = 2.50, 95% CI, 1.18-5.30); ($p = 0.02$) [64]. The authors reasoned that improving the folate status in subjects at risk of getting infected or already infected with HR-HPV may have a beneficial impact in the prevention of CC. Similar positive effects were reported for other micronutrients as well in a study analysing the role of circulating nutrients on clearance of HR-HPV infections [65]. Indeed, the likelihood of clearing HR-HPV infection was significantly higher with increasing levels of trans-lycopene ($p = 0.025$ for trend) and cis-lycopene ($p = 0.010$). The adjusted HR of the highest tertiles of trans- and cis-lycopene were 2.79 (95% CI = 1.17-6.66) and 2.92 (95% CI = 1.28-6.63) compared with the lowest tertiles. These observations suggest that higher concentrations of trans- and cis-lycopene may reduce the time to clearance of an oncogenic HPV infection [65]. Clearly, because of the great complexity of the issue, more data are needed to permit more firm conclusions on the role of nutrition in regulation of HR-HPV clearance.

Viral factors predicting HPV clearance

In addition to these implicated co-factors discussed above, several characteristics of the infecting papillomaviruses themselves are likely to be important determinants of the outcome of these infections, whether cleared or remaining persistent [3, 4, 29]. Until now, these viral factors have been mostly assessed as risk factors of viral persistence, which is by far clinically the most significant outcome. The relatively scarce literature on viral factors as modifiers of HPV clearance is briefly discussed in the following section.

Viral load

Viral load measured semi-quantitatively using HCII assay was shown to be an important independent determinant of incident Pap smear abnormalities [15] as well as persistence of HPV infections [61]. On the other hand, viral load was not shown to be related to HPV clearance in our cohort [21]. Similar results have been reported by others, while showing that the incidence of Pap smear abnormalities strongly depended on baseline viral load and HR-HPV persistence [66]. Conversely, women who were consistently HR-HPV negative or transiently HR-HPV positive, irrespective of their baseline Pap test, did not develop CIN2/3+ during follow-up. These authors suggested that a high viral load could be used as a short-term marker of progression toward precancerous lesions, although lower load does not inevitably exclude progressive disease [66].

In another study, van Duin *et al.*, studied HPV 16 viral load (real-time PCR) in cervical scrapes and development of CIN2/3 in a nested case-control study of women with normal cytology (group A) and in those with abnormal cytology (group B) [67]. In both groups, women with the 50% highest viral load had an increased relative risk of CIN2/3 and a decreased chance of both viral clearance and cytologic regression. Thus, even in women with normal cytology, an increased HPV16 load confers an increased risk of developing a CIN lesion, and a persistent high viral load is a predictor for progressive CIN [67]. Similar conclusions were reached in another recent report, where kinetics of HPV load, rather than single HPV detection, was a more reliable estimator of whether HPV infection will progress or clear [68].

Wensveen *et al.* evaluated the association between semi-quantitative viral load and cytological or histological outcome in a series of 148 women with ASCUS/AGUS cytology [69]. In their series, absence or clearance of HPV showed significantly more regression to normal cytology than persistent or newly acquired infected women. However, viral load at enrolment was not correlated with the follow-up cytological outcome, but a marked association was found between viral load and histological outcome ($p < 0.0001$). Consistent with our data [21], viral load at enrolment was not a useful predictor of cytological regression [69]. Furthermore, there was large overlapping of viral loads among the grades of CIN, and the authors stated that viral load is not a useful parameter to predict high-grade lesions in women with ASCUS/AGUS cytology [69]. Clearly, more studies are urgently needed to elucidate the role of viral load as a determinant of HPV clearance [53].

HPV types and viral integration

In several contexts above, HPV types have been listed among potential determinants of whether the infection remains persistent or undergoes spontaneous regression. Again, practically all type-specific data on outcome of HPV infections are focused on assessing type-specific HPV persistence, with scanty of information on type-specific clearance. The scarcity of reports is, at least in part, due to the fact that repeated HPV genotyping is needed to provide these data, and such an approach necessitates a prospective study design. At present, the data on the possible influence of HPV integration status on HPV clearance are even more fragmentary.

In a recent survey, Cuschieri *et al.* examined women from a screening population who had normal cytology and who were HR-HPV positive, recalled after two to three years for cytology and HPV genotyping [70]. Development of cervical neoplasia at follow-up was related to the course of HPV infection (clearance, persistence) and the presence of single or multiple HPV infections at baseline. In their cohort, women with type-specific persistent infections were significantly more likely to develop CIN than women who cleared their infection ($p = 0.0001$) or were sequentially infected with different types ($p = 0.001$). Women with multiple HPV infections at baseline were no more likely to develop abnormal PAP than those with a single infection. Clearly, type-specific persistent HR-HPV infection as monitored by genotyping can identify women at increased risk of CIN more accurately than a single or repeated presence/absence HPV test. However, the cost-effectiveness of such an approach should be investigated by a large scale cost-benefit analysis [70, 71].

As a part of our ongoing reporting of the NIS cohort data, we recently conducted a detailed assessment of the type-specific outcomes, based on prospective follow-up of almost 900 women [53]. HPV genotyping was done with real-time PCR, detecting HPV types 16, 18/45, 31, 33/52/58, 35, and 39, and the integration status of HPV16 was determined by using a novel TaqMan-based PCR method. The mean clearance time for the individual HR-HPV type-specific infection was 16.5 months (range = 0.9-34.9 months). HPV16 and HPV31 were the two most persistent infections (clearance times 18.1 and 16.2 months, respectively), whereas HPV39 infections cleared most rapidly. The mean copies per cell in HPV18/45, HPV31, HPV33/52/58, and HPV39 infections were higher in persisting HPV infections than in HPV infections that cleared, but the difference was not significant. Integration of HPV16 was not found to correlate with HPV persistence or clearance. Thus, a large proportion of women remained high-risk HPV positive for at least 18 months of follow-up. Co-infection with multiple HPV types, viral load, or integration status did not correlate with persistence/clearance of HR-HPV infections [53].

The feasibility of HPV genotyping [70] was discussed in a recent Consensus Report launched by an international expert panel during the EUROGIN 2006 Congress in Paris (April 2006) [71]. The report was concluded by stating that "as we move from cytology-based screening to HPV-based screening, genotyping may prove useful in stratifying HPV+ women according to risk of prevalent or incipient precancer and cancer to determine the appropriate clinical management strat-

egy. However, to achieve benefit to patients, the addition of HPV genotyping to cervical cancer screening must not be abused by excessive referrals to colposcopy and over-treatment, which can be exacerbated by the use of poorly-validated tests" [71]. This statement is easy to agree with. Apart from these clinical applications, HPV genotyping is clearly needed to provide additional research data on HPV-type specific outcomes in different populations.

HPV type variants

Some tentative evidence suggests that different HPV type variants might possess a different degree of inherent aggressiveness in producing progressive cervical disease [3-6, 29]. Up to today, practically nothing is known about the possible differences in clearance between the individual HPV type variants [72]. There is some fragmentary data suggesting that in a large population of HIV-infected women, the variant of HPV16 or 18 was unrelated to persistence of infection or presence of SIL. Using this reasoning, if some variant types are more oncogenic than others, such an effect is likely to become evident in a late stage in cervical carcinogenesis rather than being an early event associated with virus clearance [72].

Miscellaneous viral and host factors

The role of both humoral and cellular immunity [29, 30, 41-46] in the clearance of HPV was discussed earlier in this text, and a detailed review of the immunological mechanisms is not technically possible in this context.

Clearly, factors of both viral and host origin are needed to effectively complete the process of HPV clearance. Accordingly, Kadish *et al.* studied the role of cell-mediated immunity (e.g., lymphocyte proliferation) against HPV to identify antigenic sequences of the HPV16 proteins E6 and E7 against which an immune response may confer protection [73]. Without going into the details, they demonstrated that lymphoproliferative responses to specific HPV16 E6 and E7 peptides appeared to be associated with the clearance of HPV infection and subsequent regression of CIN [73].

The polymorphisms of the human leukocyte antigen are intimately associated with the quality of cell-mediated immune responses against all viruses. Maciag *et al.* examined whether HLA-DRB1 and HLA-DQB1 variability is related to HPV persistence [45]. They observed that DRB1*0301-DQB1*0201 haplotype was associated with a 2-fold reduction in risk for transient and persistent HPV infections, while DRB1*1102-DQB1*0301 showed a lower-risk effect only for HPV persistence. DRB1*1601-DQB1*0502 and DRB1*0807-DQB1*0402 were associated with a 7-fold and a 3-fold increase, respectively, in risk for persistence. Clearly, HLA class II polymorphisms are involved in clearance and persistence of HPV infections [45]. It is likely, however, that the basic question of why some people fail to eradicate the virus, while the vast majority do get rid of it within a relatively short time after exposure, can be satisfactorily explained only when more details are known about these HPV-specific immunological phenomena [3-5, 29, 45, 73].

DNA ploidy

In studies looking for clinical predictors of CIN lesions, analysis of DNA ploidy gained popularity in the 1980s, when several such studies were completed [4]. Finally, however, use of DNA ploidy determination proved to offer little (if anything) in the diagnosis and prediction of CIN lesions. In a more recent approach, DNA ploidy measurement was completed to assess whether DNA ploidy is of any help in resolving the discrepancies between cytology and HR-HPV testing [74]. In 497 patients who underwent repeated HPV testing, a normal DNA profile at the first smear predicted clearance of HPV infection (sensitivity, 81.5%; specificity, 45.4%; PPV, 69%; NPV, 62.4%). In persistent HR-HPV infection, a suspect DNA profile at the first smear increased the PPV from 10.8% to 22.7% for the detection of a histologically proven HSIL, with sensitivity of 95.2%. These results suggest that DNA ploidy might be useful in selecting smears with high risk of HSIL, especially among cases with persistent HR-HPV infections [74].

Biomarkers as predictors of HPV clearance

According to the recent Consensus Report of EUROGIN 2006 [75], a number of potential markers have been identified which, after clinical validation should contribute to the diagnosis, prognosis, and treatment of CC. In CC research, well characterised clinical materials including both precancer and cancer lesions enable the use of different outcome measures as dependent variables in univariate and multivariate analysis, to disclose the potential predictive factors of these outcomes of interest (disease outcome and viral events). Apart from getting new insights into the molecular pathogenesis of HPV-associated cervical carcinogenesis, we anticipate the disclosure of individual markers, a set of markers, or an expression profile of any such marker sets that would be of clinical value as predictors of disease outcome in cervical carcinogenesis and preceding HR-HPV infection [75].

Until present, however, the main research on biomarkers has been focused on assessing their performance indicators in detection of high-grade CIN lesions as well as in prognostication of CC. We were the first to study the value of biomarkers as potential predictors of outcome of cervical HR-HPV infections [76]. In our NIS cohort [22, 23], we examined the value of cell cycle regulatory proteins as predictors of the intermediate endpoint markers in cervical carcinogenesis: (a) grade of CIN, (b) HR-HPV type, (c) clearance/persistence of HR-HPV, and (d) disease outcome [76]. A series of 232 biopsy samples from women who tested HR-HPV-positive and/or with an abnormal Pap were analysed

for the following cell cycle markers: p105, p107, p130, E2F4, $p21^{CIP1/WAF1/SDI1}$, cyclin A, and Ki-67. To our major disappointment, however, none of these markers provided any useful predictive information as to the clinical and virological outcomes (clearance/persistence) during the follow-up [76]. Thus, we do not have the information yet, as to whether some of the biomarkers would be of any value in predicting HR-HPV clearance. Needless to point out, such a marker or marker panel would have a tremendous impact on clinical practices, if capable of accurately predicting the women who are likely to spontaneously resolve their HR-HPV infection.

HPV clearance in cervical lesions after treatment

As stated in the Introduction, HPV clearance as a result of local ablative or destructive treatment of cervical lesions is discussed only as far as pertinent to the role of HR-HPV in eradication or recurrence of CIN lesions [27].

In the management of CIN, one of the key issues is their adequate treatment by radical excision. This subject has been addressed in a sizeable number of reports analysing the factors predicting residual disease and recurrence after radical treatment of CIN [27]. In addition to the well recognised risk of recurrence associated with the cone margin involvement, the role of persistent HR-HPV has achieved increasing attention in the recent literature [27, 77-83]. We [27] and others [77-83] have shown that the failures of CIN treatment are intimately linked with persistent HR-HPV infection in the cervix. These data suggest that post-treatment follow-up should include both Pap smear and HPV testing for early detection of any patients at increased risk for disease recurrence and/or progression due to persistent HR-HPV types [27, 77-83]. More detailed discussion on HPV testing in the post-treatment follow-up falls outside the scope of this communication, however.

Biomarkers in prediction of HPV clearance after treatment

Taken that persistent HR-HPV infections seem to be a significant risk factor for disease recurrence as well as a sensitive and specific marker of residual disease after CIN treatment [27, 77-83], it would be of utmost importance to develop accurate means to predict HR-HPV clearance in such patients. A systematic attempt along these lines was made in another recent study of this author (the HPV-PathogenISS study), where 13 different biomarkers were tested in a well characterised series of CIN and CC lesions, as described previously in this journal [84]. A significant number of women were followed-up by serial PCR testing after treatment of their CIN lesions, which enabled the assessment of these 13 biomarkers as potential predictors of HR-HPV clearance during post-treatment follow-up.

This analysis was completed during 2004-2006, resulting in a series of 12 reports [85-96]. In these individual studies, expression of each of the following biomarkers: E-Cadherin, ERK-1, LR67, MMP-2, TIMP-2, NFkB, nm23-H1, p16INK4A, PCNA, Survivin, hTERT, Topo-2a, and VEGF-C was analysed in a series of 150 CC and 152 CIN lesions using standard immunohistochemical approaches. The predictive power of these markers was tested for four different outcomes: 1) high-grade CIN, 2) HR-HPV, 3) clearance of HR-HPV after CIN treatment, and 4) survival of CC patients, first for each individual marker in univariate analyses, followed by a meticulous modelling in multivariate analysis [97]. Several of these markers were significant predictors of a) high-grade CIN and b) HR-HPV, and few also predicted survival of CC in univariate analysis [85-96]. When entered in a multivariate model, five of these markers proved to be significant independent predictors of high-grade CIN, three markers were independent predictors of HR-HPV, and two were independent prognostic factors in CC [97]. Highly unfortunately, however, none of the tested 13 biomarkers was of any value in predicting the clearance of HR-HPV in women who were followed-up after radical treatment of their CIN lesion even in univariate analysis [85-96].

These data strongly implicate the difficulty in elaborating any single marker or even a panel of markers that would reliably forecast whether HR-HPV is likely to persist or clear in the uterine cervix following surgical treatment of CIN. The case might be different, however, when immunomodulatory treatments are used instead of surgery, as suggested by an interesting recent study where expression of MHC class I and II molecules (HLA-DR, HLA-HC and HLA-Bw4) was correlated with regression/ persistence of HR-HPV in women who received IFN-gamma treatment for their CIN1-CIN2 lesions [98]. Interestingly, up-regulation of selected MHC class I allele expression (but not MHC class II) induced by IFN-gamma was shown to closely correlate with the resolution of CIN lesions and clearance of HR-HPV DNA in these women [98]. It remains to be seen, whether any such up-regulation of the host immune system markers can be evoked by therapeutic HPV vaccines.

HPV clearance in special groups

In addition to the immunosuppressed women already discussed above, two other special groups of subjects deserve a short discussion in this context; i) newborn babies/infants, and ii) elderly (postmenopausal) women. In both groups, clearance/persistence of HR-HPV infections has potentially important clinical implications that need far more attention than paid until recently. The limited space does not allow an in-depth penetration into these special topics, however, the reader is being referred to recent comprehensive expert reviews [99-101].

Newborn babies and small children

The special issues related to HPV infections in children have received more attention only recently [99]. Until now, practically nothing has been known about the dynamics of HPV transmission among newborn babies and small children, apart from the cutaneous HPV lesions (skin warts) that have been exhaustively studied for several decades. As to the genital and oral HPV lesions, one thing seems clear by now; sexual transmission only explains a minor fraction of all HPV infections encountered in newborn babies and infants [99].

To cast further light on the previously unexplored issues on HPV transmission among newborns and infants, a special study was designed to assess the dynamics of HPV transmission within regular families, known as the Finnish HPV Family study [99]. In that prospective cohort, both parents and their newborn babies were extensively sampled for HPV testing, and prospectively followed-up for several years to explore the modes of HPV transmission between parents and their infants, as well as to elucidate the outcomes of HPV carriage during early infancy and childhood [99, 102].

In one of the recent reports from the Finnish HPV Family study, the authors analysed the data on acquisition, persistence and clearance of HR-HPV DNA in the oral and genital mucosa of these infants [102]. During the follow-up period (26.2 months), HPV DNA was found to be present in 12%-21% of oral scrape samples and in 4%-15% of genital scrape samples obtained from the infants. Oral HPV infection was acquired by 42% of children, cleared by 11%, and persisted in 10% of the infants, whereas 37% were never infected. The corresponding figures for genital HPV infection were 36%, 14%, 1.5%, and 47%. Kaplan-Meier analysis revealed that both the cumulative incidence of infection and clearance of HR-HPV were parallel at oral and genital sites [102]. Persistent oral HPV infection in the child was significantly associated with persistent oral HPV infection of the mother at month 36 of follow-up, hand warts in the mother, young age at onset of sexual activity for the mother, and the mother's use of oral contraception, as well as with the father's oral HPV status at 24 months. Persistent genital HPV infection in the infant was predicted by the mother's age at initiation of smoking at 18-21 years, and by a history of genital warts. Persistent carriage of HR-HPV types was detected in oral and genital mucosa specimens obtained from 10% and 1.5%, respectively, of the infants during their first 26 months of life.

In the near future, this unique study design hopefully will permit soliciting the answers to most of the open key questions on HPV infections in early childhood that bear important implications, e.g., in designing the population-based programmes for prophylactic HPV vaccination [99].

Elderly (postmenopausal) women

During the past few years, the dynamic model of HPV acquisition, clearance and persistence explaining the linearly declining age-specific prevalence curve [3, 4, 29] has been challenged by the data from several population-based studies, reporting a second peak in HPV prevalence among women > 55 years of age [100, 101]. Indeed, the 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 [100]. In other studies, no such U-shaped prevalence curve was established, but the shape was that of a declining linear curve.

The IARC HPV Prevalence Survey data failed to give one single explanation for these differences [101], and several key questions still remain unanswered to explain the observed increase in HPV prevalence among older women [51, 100]. One of these open questions is: Are there any age-specific differences in the outcome (persistence, progression, clearance) of HPV infections? Also this question was addressed in a recent analysis of our NIS cohort, where all 3,187 women were stratified into three age categories: i) youngest age group < 25 years (n = 1,103); ii) women between 26-55 years (n = 2,004), and iii) women past 55 years (n = 80), and analysed for epidemiological, clinical and virological determinants of their HR-HPV infections [51]. Real-time PCR was used for HPV genotyping, analysis of viral loads for HPV16, 18/45, 31, 33/52/58, 35 and 39, and for load of integrated HPV16. Several factors were identified explaining this increased HR-HPV prevalence among older women, as discussed in the original report [51]. Pertinent to our current topic, these women over 55 years of age had a higher proportion of incident infections, higher rate of viral persistence, and lower rate of HR-HPV clearance. These data suggest that in women who fail to eradicate their HR-HPV infection until menopause, selection of an integrated viral clone has taken place, driving the process towards progressing disease. Consequent to this, most of the HR-HPV infections in women > 55 years were associated with high-grade CIN or CC [51]. Clearly, many more studies are necessary to fully elucidate why this non-negligible proportion of postmenopausal women fail to eradicate their HR-HPV like the majority of women do, i.e., by the age of the menopause.

Future prospects

In the above, the topics pertinent to the clearance of HR-HPV infections in the female genital tract have been addressed focusing on the uterine cervix. Starting with a review of the historical studies on CIN lesions and more recent data on cervical HPV infections, it was concluded that the natural history of CIN is basically identical with that of HPV infections, i.e., regression, persistence and progression being the three main patterns. For CIN, these data are

straightforward, and no future natural history studies are necessary on these clinical lesions. On the other hand, the outcome of HPV infections is far more complex than simply progression, regression or persistence. These viral events follow the pattern of other viral infections, where a dynamic balance seems to exist between incident infections and virus clearance. Following a rapid accumulation of incident infections after onset of sexual activity (women < 20 years), this balance has shifted in favour of virus clearance soon after 25 years of age, which explains the constantly declining age-specific prevalence of HPV infections until menopause. Failure to eradicate the virus at postmenopause is not uncommon, however, explaining the deep second peak in HPV prevalence now reported in many different populations.

There is little doubt that the mechanistic explanation for HPV clearance is by specific immunological reactions, where competent humoral and cell-mediated immune mediators are needed. Nonetheless to understand this process in detail still necessitates a substantial amount of clinical and laboratory research.

The importance of HPV clearance/non clearance (= persistence) has been well recognised, and an increasing number of studies addressing these issues have accumulated during the past few years. The data are now rather unanimous as regards the times and rates of HPV acquisition, persistence and clearance. On the other hand, data are still incomplete and in part inconsistent as to the cofactors that affect HR-HPV clearance. Until present, a wide variety of such cofactors have been explored as potential determinants and/or predictors of HPV clearance, as reviewed in this communication. Particularly disappointing have been the few studies attempting to identify suitable biomarkers as such predictors, but in most part, this is an unexplored area as yet.

Similarly, data on the two extremes of life, i.e., early infancy and postmenopause, are still far too fragmentary to enable creating a comprehensive view, how these viral infections transmit and behave in early life, and what makes some women incapable of clearing their virus at postmenopause. Both issues are of utmost importance and have widespread clinical implications. We need to know how and why some infants and children contract HR-HPV infections well before the onset of their sexual activity, to be able to select the proper targets for prophylactic HPV vaccination. Similarly, we need to know why some women are likely to remain HPV carriers after 55 years of age, while the vast majority successfully clear the infection well before the menopausal age. Early detection of cervical cancer precursors among these elderly HR-HPV positive women past the usual age of organised screening remains a major challenge also in the future.

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