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Longitudinal outcomes of high-risk human papillomavirus (HPV) infections as competing-risks events following cervical HPV test at baseline visit in the *NIS-LAMS** cohort

K. Syrjänen[1], I. Shabalova[2], L. Sarian[3], P. Naud[4], A. Longatto-Filho[5], S. Derchain[3], V. Kozachenko[2], S. Zakharchenko[6], C. Roteli-Martins[7], R. Nerovjna[8], L. Kljukina[9], S. Tatti[10], M. Branovskaja[11], M. Branca[12], V. Grunjberga[13], M. Eržen[14], A. Juschenko[13], L. Serpa Hammes[4], J. Podistov[15], S. Costa[16], S. Syrjänen[17] and the NIS* and LAMS Study Research Groups**

[1]Department of Oncology & Radiotherapy, Turku University Hospital, Turku (Finland); [2]Russian Academy of Post-Graduate Medical Education, Moscow (Russia); [3]Universidade Estadual de Campinas, Campina (Brazil); [4]Hospital de Clinicas de Porto Alegre, and Department of Gynecology and Obstetrics, Federal University of Rio Grande do Sul, Porto Alegre (Brazil); [5]Laboratory of Medical Investigation (LIM14), Department of Pathology, Faculty of Medicine, São Paulo University, São Paulo University, São Paulo (Brazil); and Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga (Portugal); [6]Novgorod Municipal Dermato-venereological Dispensary, Department of Gynaecology, Novgorod (Russia); [7]Hospital Leonor M de Barros, Sao Paulo (Brazil); [8]Novgorod Female Consultative Outpatient Hospital, Department of Gynaecology, Novgorod (Russia); [9]Research Institute of Oncology and Medical Radiology, Republican Centre of Clinical Cytology, Minsk, Belarus; [10]First Chair Gynecology Hospital de Clinicas, Buenos Aires (Argentina); [11]Minsk State Medical Institute, Department of Gynaecology and Obstetrics, Minsk (Belarus); [12]Unit of Cytopathology, National Centre of Epidemiology, Surveillance and Promotion of Health, National Institute of Health (ISS), Rome (Italy); [13]Latvian Cancer Centre, Department of Gynaecology, and Laboratory of Cytology, Riga (Latvia); [14]SIZE Diagnostic Center, Ljubljana (Slovenia); [15]N.N. Blokhin Cancer Research Centre of Russian Academy of Medical Sciences (RAMS), Moscow (Russia); [16]Department of Obstetrics and Gynecology, S. Orsola-Malpighi Hospital, Bologna (Italy); [17]Department of Oral Pathology, Institute of Dentistry, University of Turku (Finland)

Summary

Background: The complex natural history of human papillomavirus (HPV) infections following a single HPV test can be modeled as competing-risks events (i.e., no-, transient- or persistent infection) in a longitudinal setting. The covariates associated with these competing events have not been previously assessed using competing-risks regression models. *Objectives:* To gain further insights in the outcomes of cervical HPV infections, we used univariate- and multivariate competing-risks regression models to assess the covariates associated with these competing events. *Study Design and Methods:* Covariates associated with three competing outcomes (no-, transient- or persistent HR-HPV infection) were analysed in a sub-cohort of 1,865 women prospectively followed-up in the NIS (n = 3,187) and LAMS Study (n = 12,114). *Results:* In multivariate competing-risks models (with two other outcomes as competing events), permanently HR-HPV negative outcome was significantly predicted only by the clearance of ASCUS+ Pap during FU, while three independent covariates predicted transient HR-HPV infections: i) number of recent (< 12 months) sexual partners (risk increased), ii) previous Pap screening history (protective), and history of previous CIN (increased risk). The two most powerful predictors of persistent HR-HPV infections were persistent ASCUS+ Pap (risk increased), and previous Pap screening history (protective). In pair-wise comparisons, number of recent sexual partners and previous CIN history increase the probability of transient HR-HPV infection against the HR-HPV negative competing event, while previous Pap screening history is protective. Persistent ASCUS+ Pap during FU and no previous Pap screening history are significantly associated with the persistent HR-HPV outcome (compared both with i) always negative, and ii) transient events), whereas multiparity is protective. *Conclusions:* Different covariates are associated with the three main outcomes of cervical HPV infections. The most significant covariates of each competing events are probably distinct enough to enable constructing of a risk-profile for each main outcome.

Key words: HPV; Natural history; Outcomes; Competing events; Competing-risks regression model; Transient infection; Persistent infection; Prospective follow-up; NIS Cohort; LAMS Study.

*NIS, New Independent States of the Former Soviet Union; **LAMS, Latin American Screening Study.

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Introduction

The natural history of human papillomavirus (HPV) infections in the uterine cervix is closely linked with the development of cervical intraepithelial neoplasia (CIN), with three outcomes: regression, persistence and progression [1-5]. From a strictly virological standpoint, however, the natural history of cervical HPV infections is much more complex, at least six possible outcomes being distinguished in long-term longitudinal cohort studies: incident infections, early clearance, persistence, fluctuation, late clearance, and recurrence [6-11].

These early observations have been refined by more recent studies on the dynamics of cervical HPV infections at genotype level [12-14]. According to these data, incident high-risk (HR) HPV infections are clearly age-dependent, the 3-year cumulative incidence exceeding 50% after the onset of sexual activity [13-16]. On the other hand, clearance of the virus does not show such strict age-dependence [17], but continues at a rather constant rate from age 30 years onwards when the clearance rates exceed the acquisition rates, resulting in progressively declining age-specific prevalence rates [18-20]. However, not all HPV infections will undergo spontaneous clearance; a substantial proportion of acquired HR-HPV infections remain persistent [19-23]. These persistent infections by the HR-HPV genotypes are considered as prerequisite for developing a progressive CIN and eventually invasive cervical cancer (CC) if left unnoticed [24-29].

To identify the risk factors (covariates) for these optional outcomes of cervical HR-HPV infections is of utmost importance, on one hand, to avoid unnecessary therapies for transient infections that are likely to clear spontaneously, and on the other hand, to detect persistent HR-HPV infections predisposing the patient to an increased risk of developing progressive disease [30, 31]. Until now, most of the studies assessing the covariates associated with these different HPV outcomes have been based on cohort (or cross-sectional) case-control settings, where the outcome of interest (i.e., HPV acquisition, persistence, clearance) has been compared with the controls lacking this event; no acquisition, clearance, no clearance, respectively [12-29]. The most commonly used statistical techniques include conventional survival analysis (Kaplan-Meier or Cox) or Poisson regression [16, 23], while marginal (e.g., GEE, generalized estimating equation) [32] and mixed-effects models have been rarely used, despite their particular suitability for modeling this type of longitudinal data [33]. Yet, another recently introduced statistical technique known as competing-risks regression [34, 35] has been completely neglected in HPV natural history studies so far.

The outcomes of HPV infection can be considered as competing-risks events, being different for HPV-negative and HPV-positive women. Any woman testing HPV-negative is at risk for two competing events when subjected to longitudinal follow-up: i) remain permanently HPV-negative, or ii) develop an incident infection [13-16]. Similarly, baseline HPV-positive women can experience two competing events: i) clearance of the prevalent HPV, or ii) develop a persistent infection [18-29]. Because both incident and prevalent HPV infection can remain persistent or undergo spontaneous clearance, actually only three competing outcomes exist: 1) permanently HPV-negative; 2) transient HPV infection, and 3) persistent HPV infection [8, 11, 31]. These are mutually exclusive, and as such fulfill the criteria of competing-risks events amenable for modeling by the competing-risks regression [34, 35].

The present study is the first where this novel technique is used to model the covariates associated with these three competing-risks events, tested in a sub-cohort of 1,865 women prospectively followed-up in our NIS and LAMS cohort studies [15, 19, 20, 25].

Material and Methods

The NIS and the LAMS Cohort Study

The present analysis is based on the combined cohort of the NIS and the LAMS studies described in recent reports. Both studies are international multi-centre trials testing optional screening tools in three NIS (New Independent States of the Former Soviet Union) countries (Russia, Belarus and Latvia) [36] as well as in two Latin American countries (Brazil and Argentina) [36]. The design and baseline data of both cohorts have been previously detailed [36, 37].

Patients and Study Design

The material of *the NIS study* cohort comprises 3,187 consecutive women attending six different outpatient clinics in the three NIS countries between 1998 and 2002. These women derived from three different groups: i) cervical cancer screening (= SCR patients); ii) attendants of gynaecologic outpatient clinics (= GYN patients), and iii) patients examined at STD clinics (= STD patients). The mean age of these women at enrolment was 32.6 (± 10.7 SD) years (median 30.6, range 15-85 years) [36]. The study design has been detailed in a series of reports [15, 19, 20, 25]. All eligible women had Pap smear taken and were tested for HR-HPV using HC2 and the first 1,500 women also with PCR and hybridisation. Patients with ASC-US or higher Pap had biopsy confirmation at baseline [15, 19, 20, 25, 36].

The LAMS study is a longitudinal cohort of women enrolled in regions with low, intermediate, and high incidence of CC in Brazil and Argentina [37]. A total of 12,114 women were enrolled by the four clinics. The mean age of these women at enrolment was 37.9 years (median 37.7, range 14-67). In this trial, eight different diagnostic tests were compared as follows: cervical cytology (conventional Pap and LBC) was compared with i) four optional screening tools suggested for low-resource settings: a) visual inspection with acetic acid (VIA), b) visual inspection with Lugol iodine (VILI), c) cervicography, d) screening colposcopy; and ii) with the

new molecular diagnostic tools (HPV testing by Hybrid Capture 2; HC2), performed a) in samples collected by physicians, and b) in those collected by self-sampling devices [37-40]. Women testing positive with any of these techniques were examined by colposcopy.

Prospective Follow-up

Prospective follow-up (FU) is an essential component of both studies. In the NIS cohort, all women who presented with biopsy-confirmed low-grade lesions were assigned for FU, while high-grade lesions were treated. FU data are available for 887 women, of whom 33 patients with baseline CIN3 were excluded from this analysis, leaving 854 women in the final prospective NIS cohort. The mean FU time is 17.2 mo (SD, 11.6 mo; median, 16.6 mo; range 1-43 mo) [15, 19, 20, 25].

In the LAMS study, the same criteria were used to allocate the women into the FU and treatment groups [37-40]. A total of 1,011 women completed at least one FU visit, scheduled at 6-month intervals. The mean FU time is 21.7 mo (SD, 8.09 mo; median, 24.2 mo; range 1-54 mo). All high-grade lesions were promptly treated and followed-up for the same period, using repeated Pap test and colposcopy at 6-month intervals, and HC2 assay at 12-month intervals.

Methods

Because the methods used in these two cohort studies are detailed in several reports [15, 19, 20, 25, 36-40], they are described here only as far as pertinent to elaborating the data used in the present analysis.

Recording the risk factors by questionnaire

In both studies, all women who gave their consent to participate filled in a detailed inquiry concerning the risk factors of HPV, CIN and CC. In combining the two databases, only the variables that were recorded in both cohorts were maintained to make the data consistent. The present analysis is based on the following variables recorded at baseline: age, marital status, years of education, race, age at first sexual intercourse, number of pregnancies, -live births, -abortions, number of life-time sexual partners, number of sexual partners during the past 12 months, partners' STD history, mode of contraception, years of hormonal contraception, history of STDs, previous Pap screening history, history of CIN, history of genital warts, and smoking history [36, 37, 41].

Papanicolaou (Pap) smears

In the NIS study, all women were examined using conventional Pap smears [36], whereas in the LAMS study, three methods were used: conventional PAP and two different LBC techniques (DNA-Citoliq; Digene Brazil, Sao Paulo, and SurePath; TriPath, Durham, NC, USA) [38]. In the present analysis, only the results of the conventional Pap test were used (available from all patients).

Directed Punch Biopsy

Directed punch biopsies (and cones) were fixed in formalin, embedded in paraffin, and processed into 5- m-thick hematoxylin-eosin (HE)-stained sections for light microscopy, following the routine procedures. All biopsies were examined among the daily routine in the Pathology Departments of the partner institutions, and diagnosed using the commonly agreed CIN nomenclature.

Detection of HR-HPV DNA by Hybrid Capture 2 assay

In both studies, the principal HPV testing method was Hybrid Capture 2 (HC2) assay, performed using cervical swabs (collected by a physician) or self-sampling devices (tampons, in LAMS study only), as described previously [36,37,40). HC2 assay (n = 3,084 baseline tests in the NIS and n = 4,694 in the LAMS) was performed using the automated HC2 test system according to the manufacturer's protocol. The samples were analysed only for the presence of HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. Samples were classified as HR-HPV positive with the RLU/CO ≥ 1.0 pg/ml cut-off.

HPV outcomes and competing-risks events

The 854 women from the NIS cohort and 1,011 women from the LAMS study were merged into the same database in the panel format, clustered by woman-ID and FU-visits as the time variable. The combined cohort of 1,865 women was first categorised according to all possible outcomes of their baseline cervical HPV infections during the follow-up, as follows: 1) permanently HR-HPV negative (n = 326), 2) incident HR-HPV infection (n = 59), 3) persistent (prevalent) HR-HPV infection (n = 368), 4) clearance of (prevalent) HR-HPV infection (n = 496), 5) HC2 test not done (n = 153), 6) only one (positive) HC2 test available (n = 270), 7) fluctuating course of HR-HPV infection (n = 63), and 8) only one (negative) HC2 test available (n = 130).

In the next step, these three competing-risks events (always negative, transient, persistent) were defined among those eight outcome categories, following the principles detailed recently [42]. Women testing invariable HC2 negative throughout the follow-up period were included in the first category (permanently HR-HPV negative). The second category (transient HR-HPV infection) comprises all women, among whom either incident (new infection) or prevalent (baseline) HR-HPV infection cleared by the end of FU. All women in whom either prevalent (baseline) or incident HR-HPV infection persisted for at least six months (6M+) [42] were classified as having the third competing event (persistent HR-HPV infection). As described before, inclusion into this category necessitates that the infection persists also in the last FU-visit [42]. Similarly, all women with only one (or no) HC2 assay available, were omitted from the analysis. In the panel data format, all these events were recorded at each FU visit, resulting in a panel with 969 HC2-negative records (for category one women only), 1024 events for 6M+ persistent infections, and 580 (clearance) events for all transient infections.

Table 1. — *Covariates associated with the three HR-HPV outcomes in an univariate competing-risks regression model (with the other two as competing events).*

HPV covariate	Permanently HR-HPV negative* (vs persistent and transient HPV)		Persistent HR-HPV infection** (vs HPV-negative and transient HPV)		Transient HR-HPV infection*** (vs HPV-negative and persistent HPV)	
	SHR (95% CI)	*p*	SHR (95% CI)	*p*	SHR (95% CI)	*p*
Cohort:						
NIS	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
LAMS	1.19 (1.02-1.38)	0.025	0.46 (0.37-0.56)	0.0001	0.75 (0.64-0.89)	0.001
CC incidence region:						
Low-incidence region	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Intermediate-incidence region	1.09 (0.94-1.27)	0.217	1.05 (0.84-1.31)	0.639	0.88 (0.74-1.04)	0.156
High-incidence region	0.27 (0.18-0.39)	0.0001	1.17 (0.88-1.55)	0.272	0.66 (0.50-0.87)	0.004
Age:						
Above 30 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Below 30 years	0.67 (0.57-0.78)	0.0001	2.06 (1.68-2.539	0.0001	1.32 (1.13-1.55)	0.0001
Marital status:						
Living with partner	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Single						
Baseline Pap test ASCUS+:						
PAP negative	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
PAP ASCUS+	1.15 (0.99-1.33)	0.057	1.61 (1.32-1.96)	0.0001	0.97 (0.82-1.139	0.732
Pap test follow-up:						
Incident ASCUS+ Pap (yes/no)	0.58 (0.47-0.72)	0.0001	1.34 (1.06-1.70)	0.012	0.99 (0.82-1.21)	0.994
Persistent ASCUS+ Pap (yes/no)	0.81 (0.63-1.04)	0.102	2.50 (1.89-3.30)	0.0001	1.13 (0.88-1.45)	0.317
ASCUS+ Pap cleared (yes/no)	1.23 (1.05-1.44)	0.008	0.85 (0.67-1.06)	0.149	0.95 (0.79-1.13)	0.570
Years of education:						
More than 11 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 8-11 years	0.83 (0.60-1.16)	0.289	1.16 (0.68-1.96)	0.569	1.03 (0.71-1.51)	0.841
Between 5-8 years	0.97 (0.68-1.36)	0.848	0.91 (0.51-1.64)	0.776	1.14 (0.77-1.70)	0.487
Less than 5 years	0.99 (0.70-1.39)	0.970	0.99 (0.57-1.74)	0.992	0.82 (0.54-1.24)	0.354
Race:						
White	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Non-white (black, mixed, other)	1.10 (0.91-1.31)	0.304	0.56 (0.41-0.77)	0.0001	1.06 (0.86-1.30)	0.541
Age at onset of sexual activity:						
At or above 20 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 17 and 20 years	0.88 (0.73-1.04)	0.156	1.03 (0.80-1.33)	0.779	0.94 (0.77-1.15)	0.576
Between 15 and 17 years	0.85 (0.69-1.04)	0.130	1.23 (0.92-1.64)	0.159	0.99 (0.79-1.25)	0.982
Below 15 years	0.98 (0.74-1.29)	0.906	0.92 (0.58-1.46)	0.749	1.33 (0.99-1.79)	0.055
Ever been pregnant:						
No	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Yes	1.40 (1.17-1.70)	0.0001	0.67 (0.54-0.85)	0.001	0.76 (0.63-0.90)	0.002
Number of pregnancies:						
0	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1	1.42 (1.12-1.80)	0.003	0.82 (0.62-1.10)	0.199	0.89 (0.71-1.12)	0.361
2	1.48 (1.17-1.85)	0.001	0.71 (0.53-0.96)	0.026	0.76 (0.60-0.97)	0.031
3	1.39 (1.08-1.78)	0.009	0.66 (0.47-0.92)	0.015	0.74 (0.57-0.96)	0.025
4 or more	1.38 (1.10-1.72)	0.004	0.52 (0.37-0.73)	0.0001	0.66 (0.52-0.84)	0.001
Number of live births:						
0	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1	1.20 (1.01-1.44)	0.049	1.00 (0.79-1.28)	0.959	0.83 (0.69-1.01)	0.064
2	1.23 (1.01-1.52)	0.042	0.97 (0.72-1.29)	0.839	0.70 (0.55-0.90)	0.007
3 or more	1.02 (0.81-1.27)	0.855	0.47 (0.31-0.70)	0.0001	0.63 (0.48-0.84)	0.001
Number of life-time sexual partners:						
1	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
2-3	0.78 (0.63-0.97)	0.030	1.51 (1.01-2.27)	0.042	1.34 (1.01-1.78)	0.041
4-5	0.72 (0.54-0.97)	0.033	1.54 (0.94-2.51)	0.085	1.17 (0.83-1.66)	0.353
6 or more	0.90 (0.62-1.31)	0.601	2.69 (1.63-4.45)	0.0001	1.59 (1.06-2.39)	0.027
Number of partners during past 12 months:						
0	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1	0.94 (0.66-1.33)	0.745	1.03 (0.63-1.69)	0.902	1.45 (0.93-2.25)	0.093
2 or more	0.70 (0.47-1.06)	0.085	2.03 (1.20-3.43)	0.008	1.96 (1.23-3.13)	0.005
Any sexual partner with diagnosed STD:						
No	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Yes	0.90 (0.70-1.15)	0.420	1.15 (0.83-1.58)	0.384	1.09 (0.83-1.43)	0.519

following Table 1. — *Covariates associated with the three HR-HPV outcomes in an univariate competing-risks regression model (with the other two as competing events).*

HPV covariate	Permanently HR-HPV negative* (vs persistent and transient HPV)		Persistent HR-HPV infection** (vs HPV-negative and transient HPV)		Transient HR-HPV infection*** (vs HPV-negative and persistent HPV)	
	SHR (95% CI)	*p*	SHR (95% CI)	*p*	SHR (95% CI)	*p*
Mode of contraception:						
No contraception	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Oral contraception	1.13 (0.93-1.38)	0.205	0.81 (0.63-1.05)	0.115	1.05 (0.84-1.30)	0.640
Other contraception	1.07 (0.89-1.29)	0.439	0.64 (0.50-0.84)	0.001	1.06 (0.87-1.319	0.539
Oral contraception:						
Never	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Ever (current and past)	1.08 (0.93-1.27)	0.305	1.04 (0.83-1.29)	0.715	1.01 (0.85-1.20)	0.890
History of STD:						
Never	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Ever	0.93 (0.75-1.17)	0.572	1.24 (0.93-1.67)	0.136	1.22 (0.97-1.53)	0.080
Previous Pap smear taken:						
Never	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Ever	1.11 (0.94-1.32)	0.197	0.59 (0.48-0.74)	0.0001	0.69 (0.58-0.82)	**0.0001**
Time since last Pap smear:						
More than 24 months	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 12 and 24 months	1.07 (0.80-1.43)	0.620	0.78 (0.48-1.36)	0.322	0.82 (0.59-1.15)	0.267
Between 6 and 12 months	0.86 (0.66-1.13)	0.294	0.76 (0.49-1.16)	0.210	0.74 (0.54-1.01)	0.059
Less than 6 months	0.89 (0.66-1.21)	0.492	1.10 (0.71-1.70)	0.649	0.94 (0.68-1.30)	0.745
History of previous CIN:						
No	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Yes	1.12 (0.83-1.52)	0.438	0.73 (0.41-1.27)	0.264	1.60 (1.20-2.14)	**0.001**
Ever been smoker:						
Never	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Ever (current and past)	0.93 (0.78-1.10)	0.403	0.92 (0.72-1.18)	0.537	0.97 (0.80-1.17)	0.754
Duration of smoking:						
Less than 5 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 5 and 10 years	1.31 (0.98-1.76)	0.061	0.81 (0.55-1.22)	0.327	0.96 (0.70-1.30)	0.799
Longer than 10 years	1.36 (1.02-1.81)	0.035	0.66 (0.44-0.97)	0.036	0.84 (0.61-1.17)	0.308

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; SHR, subhazard ratio; NC, not computable.

Statistical analyses

All statistical analyses were performed using the SPSS 19.0.1. for Windows (IBM, NY, USA) and STATA/SE 12.0 software (STATA Corp., Texas, USA). This longitudinal data file was constructed into a panel format, clustered by woman-ID and using the FU-visits as the time (repeated measures) variable. Competing-risks regression models (STATA strcegg) [34, 35] were first used in univariate mode to estimate the risk, i.e., crude subhazard ratios (SHR and 95% CI) of different covariates to associate with i) permanently HR-HPV negative outcome, ii) transient HR-HPV outcome, and iii) persistent HR-HPV outcome. Multivariate models were constructed to disclose independent covariates, calculating SHRs (95% CI) adjusted for age and all significant univariates. These analyses were repeated for all three competing events, using the other outcomes as competing-risks events [34, 35]. To enable pair-wise comparisons between the outcomes, the competing-risks event in the model was changed appropriately, i.e., transient HPV vs always negative, persistent HPV vs always negative, and persistent HPV vs transient HPV. In all calculations, robust variance estimator (vce) was used, clustered by woman-ID, to account for the repeated sampling of each woman. Woman-ID was not set as an ID-variable, however, because we wanted the STATA strcegg to treat each observation within individual patients as a distinct spell, not as a set of overlapping spells. All tests were 2-sided, and values $p < 0.05$ were regarded as statistically significant.

Results

Table 1 summarizes the results of univariate competing-risks regression analysis of all recorded covariates associated with the three endpoints. Keeping the other two outcomes as competing-risks events in the model, the covariates associated with each of the three outcomes (always negative, transient HPV, persistent HPV) are quite distinct and show different predictive power (SHRs).

The most powerful cofactors (at p = 0.0001 level) of permanently HR-HPV negative outcome include i) high-incidence region for CC (negative association), ii) age below 30 years (negative association), iii) incident ASCUS+ Pap smear during FU (negative association), iv) ever pregnant (positive association). Another significant covariates include a) clearance of ASCUS+ Pap during FU, b) parity variables (pregnancies and live births)(positive association), c) number of life-time sexual partners.

Table 2. — *Significant covariates associated with the three HR-HPV outcomes in multivariate competing-risks regression model (with the other two as competing events).*

Competing outcome events	@Adjusted SHR	95% CI		Significance
		Lower Bound	Upper Bound	
*Permanently HR-HPV negative**				
Cohort[1]	omitted			
CC incidence region	0.91	0.69	1.20	0.542
Age (30-yrs cut-off)	0.89	0.58	1.37	0.627
Incident ASCUS+ Pap	0.74	0.45	1.21	0.238
ASCUS+ Pap cleared	**1.61**	**1.09**	**2.37**	**0.015**
Ever been pregnant	1.74	0.94	3.25	0.078
Number of pregnancies	0.93	0.73	1.18	0.584
Number of live births	0.94	0.76	1.16	0.592
Number of life-time sexual partners	1.02	0.85	1.23	0.818
Duration of smoking	1.05	0.87	1.28	0.597
*Persistent HR-HPV Infection***				
Cohort[1]				
Age (30-yrs cut-off)	1.59	0.69	3.64	0.268
Baseline ASCUS+ Pap[1]	omitted			
Incident ASCUS+ Pap[1]	omitted			
Persistent ASCUS+ Pap	**8.85**	**2.75**	**28.42**	**0.0001**
Race	0.88	0.37	2.06	0.771
Ever been pregnant	0.53	0.14	1.95	0.345
Number of pregnancies	**0.34**	**0.14**	**0.79**	**0.012**
Number of live births	0.51	0.22	1.16	0.113
Number of life-time sexual partners	1.09	0.68	1.73	0.715
Number of recent (< 12 months) sexual partners	1.05	0.16	6.86	0.953
Mode of contraception	0.92	0.52	1.63	0.790
Previous Pap taken (screening history)	**0.06**	**0.01**	**0.33**	**0.0001**
Duration of smoking[1]	omitted			
*Transient HR-HPV Infection****				
Cohort[1]	omitted			
CC incidence region[1]	omitted			
Age (30-yrs cut-off)	1.08	0.87	1.43	0.444
Ever been pregnant	0.89	0.66	1.19	0.441
Number of pregnancies	0.92	0.81	1.04	0.207
Number of live births	1.00	0.87	1.14	0.967
Number of life-time sexual partners	1.12	0.98	1.27	0.079
Number of recent (< 12 months) sexual partners	**1.56**	**1.23**	**2.45**	**0.038**
Previous Pap taken (screening history)	**0.71**	**0.58**	**0.88**	**0.002**
History of previous CIN	**1.72**	**1.25**	**2.37**	**0.001**

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; @Adjusted for all covariates that were significant in univariate model; 1Omitted from the model because of collinearity; SHR, subhazard ratio; significant covariates are in bold.

Only two very powerful ($p = 0.0001$) covariates were associated with transient HR-HPV infections; i) age below 30 years, and ii) previous Pap screening history (protective). Several others reached $p = 0.001$ significance level, including the cohort itself (LAMS negative), parity variables (multiple protective), and history of previous CIN (increasing the risk). Yet several other covariates showed an association, with lower significance levels.

A long list of covariates are associated with persistent HR-HPV infections at the $p = 0.0001$ significance level. These include: i) LAMS cohort (protective), ii) age below 30 years (risk increased), iii) baseline ASCUS+ Pap smear (increase), iv) persistent ASCUS+ Pap, v) race (non-white protective), vi) multiparity (pregnancies/deliveries)(protecting), vii) number of life-time sexual partners (positive), and viii) previous Pap screening history (protective). Several other significant covariates were disclosed, with lower significance levels (Table 1).

When all these significant covariates of the univariate analysis were entered in multivariate competing-risks regression models, only a few remained significant independent predictors of each HPV outcome *(Table 2)*. Permanently HR-HPV negative outcome was significantly predicted only by the clearance of ASCUS+ Pap during FU ($p = 0.015$). There were three independent covariates of transient HR-HPV infections: i) number of recent (< 12 months) sexual partners (increase the risk), ii) previous Pap screening history (protective), and history of previous CIN (increased risk). The single most powerful predictor of persistent HR-HPV infections was persistent ASCUS+ Pap during FU, with SHR = 8.85 (95% CI 2.75-28.42) ($p = 0.0001$). Equally powerful but to another direction (protective) was previous Pap screening history, with SHR = 0.06 (95% CI 0.01-0.33) ($p = 0.0001$). Also increased parity was an independent covariate, protective against the persistent outcome ($p = 0.012$).

Table 3. — *Significant covariates associated with transient and persistent HR-HPV outcomes in univariate competing-risks regression model (with permanently HR-HPV-negative and transient HR-HPV infections as competing events, respectively).*

HPV covariate	Transient HR-HPV infection*** (vs permanently HR-HPV negative)		Persistent HR-HPV infection** (vs permanently HR-HPV negative)		Persistent HR-HPV infection** (vs transient HR-HPV infection)	
	SHR (95% CI)	*p*	SHR (95% CI)	*p*	SHR (95% CI)	*p*
Cohort:						
NIS	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
LAMS	0.73 (0.61-0.85)	**0.0001**	0.46 (0.37-0.56)	**0.0001**	0.48 (0.38-0.599	**0.0001**
CC incidence region:						
Low-incidence region	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Intermediate-incidence region	0.88 (0.74-1.059	0.188	1.04 (0.83-1.30)	0.695	1.06 (0.85-1.32)	0.588
High-incidence region	0.64 (0.48-0.85)	**0.002**	1.11 (0.84-1.49)	0.438	1.04 (0.78-1.39)	0.766
Age:						
Above 30 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Below 30 years	1.42 (1.21-1.67)	**0.0001**	2.11 (1.71-2.59)	**0.0001**	2.00 (1.62-2.47)	**0.0001**
Baseline Pap test ASCUS+:						
PAP negative	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
PAP ASCUS+	1.01 (0.85-1.18)	0.891	1.60 (1.31-1.95)	**0.0001**	1.62 (1.33-1.98)	**0.0001**
Pap test follow-up:						
Incident ASCUS+ Pap (yes/no)	1.02 (0.83-1.24)	0.847	1.34 (1.05-1.69)	**0.015**	1.27 (1.01-1.62)	**0.041**
Persistent ASCUS+ Pap (yes/no)	1.34 (1.05-1.73)	**0.020**	2.58 (1.94-3.43)	**0.0001**	2.53 (1.90-3.37)	**0.0001**
ASCUS+ Pap cleared (yes/no)	0.91 (0.76-1.09)	0.340	0.83 (0.66-1.04)	0.118	0.85 (0.67-1.06)	0.161
Race:						
White	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Non-white (black, mixed, other)	1.04 (0.84-1.27)	0.743	0.57 (0.41-0.77)	**0.0001**	0.57 (0.42-0.78)	**0.0001**
Age at onset of sexual activity:						
At or above 20 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 17 and 20 years	0.94 (0.77-1.16)	0.592	1.02 (0.79-1.32)	0.823	1.01 (0.78-1.31)	0.885
Between 15 and 17 years	1.04 (0.82-1.32)	0.733	1.24 (0.92-1.66)	0.147	1.23 (0.91-1.65)	0.162
Below 15 years	1.36 (1.01-1.85)	**0.042**	0.95 (0.60-1.51)	0.859	0.94 (0.59-1.49)	0.809
Ever been pregnant:						
No	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Yes	0.71 (0.59-0.86)	**0.0001**	0.65 (0.52-0.82)	**0.0001**	0.68 (0.54-0.86)	**0.001**
Number of pregnancies:						
0	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1	0.86 (0.68-1.10)	0.245	0.81 (0.60-1.08)	0.167	0.84 (0.63-1.13)	0.263
2	0.72 (0.56-0.93)	**0.012**	0.69 (0.51-0.93)	**0.017**	0.73 (054-0.98)	**0.040**
3	0.69 (0.53-0.91)	**0.009**	0.64 (0.46-0.89)	**0.010**	0.67 (0.48-0.93)	**0.020**
4 or more	0.60 (0.47-0.77)	**0.0001**	0.50 (0.35-0.69)	**0.0001**	0.52 (0.37-0.73)	**0.0001**
Number of live births:						
0	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1	0.82 (0.67-0.99)	**0.046**	0.98 (0.77-1.26)	0.900	1.01 (0.79-1.28)	0.926
2	0.69 (0.53-0.90)	**0.006**	0.94 (0.70-1.27)	0.704	0.98 (0.72-1.32)	0.899
3 or more	0.59 (0.44-0.78)	**0.0001**	0.46 (0.30-0.68)	**0.0001**	0.46 (0.31-0.69)	**0.0001**
Number of life-time sexual partners:						
1	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
2-3	1.37 (1.03-1.83)	**0.031**	1.53 (1.02-2.29)	**0.038**	1.48 (0.99-2.22)	0.055
4-5	1.21 (0.85-1.72)	0.277	1.55 (0.94-2.54)	0.083	1.50 (0.91-2.48)	0.105
6 or more	1.77 (1.17-2.68)	**0.007**	2.80 (1.69-4.65)	**0.0001**	2.78 (1.68-4.58)	**0.0001**
Number of partners during past 12 months:						
0	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
1	1.48 (0.94-2.32)	0.084	1.05 (0.64-1.73)	0.831	1.03 (0.63-1.70)	0.884
2 or more	2.17 (1.33-3.49)	**0.002**	2.12 (1.25-3.61)	**0.005**	1.99 (1.17-3.38)	**0.011**
Mode of contraception:						
No contraception	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Oral contraception	1.05 (0.84-1.30)	0.674	0.82 (0.63-1.06)	0.148	0.85 (0.65-1.09)	0.198
Other contraception	1.03 (0.83-1.279	0.803	0.65 (0.50-0.84)	**0.001**	0.65 (0.51-0.85)	**0.001**
Previous Pap smear taken:						
Never	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Ever	0.66 (0.55-0.79)	**0.0001**	0.58 (0.47-0.739	**0.0001**	0.60 (0.48-0.76)	**0.0001**
Time since last Pap smear:						
More than 24 months	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 12 and 24 months	0.82 (0.58-1.14)	0.249	0.77 (0.48-1.25)	0.308	0.79 (0.49-1.27)	0.343
Between 6 and 12 months	0.71 (0.51-0.979	**0.033**	0.73 (0.48-1.13)	0.163	0.72 (0.47-1.12)	0.143
Less than 6 months	0.94 (0.68-1.319	0.737	1.09 (0.70-1.68)	0.698	1.06 (0.68-1.64)	0.771
History of previous CIN:						
No	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Yes	1.58 (1.16-2.14)	**0.003**	0.75 (0.42-1.32)	0.324	0.73 (0.41-1.29)	0.285
Duration of smoking:						
Less than 5 years	1.00 (Reference)		1.00 (Reference)		1.00 (Reference)	
Between 5 and 10 years	0.96 (0.70.1.31)	0.803	0.82 (0.55-1.23)	0.346	0.85 (0.57-1.27)	0.434
Longer than 10 years	0.82 (0.59.1.14)	0.252	0.65 (0.44-0.96)	**0.034**	0.68 (0.45-1.00)	0.053

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; SHR, subhazard ratio; NC, not computable.

The three HPV outcomes are compared pair-wise for their covariates by changing the competing-risks events in the models. Only significant covariates are listed in *Table 3*. It is obvious that several covariates make distinction between transient HR-HPV outcome and permanently HR-HPV negative outcome, of which six do so at the $p = 0.0001$ level. The covariate profile is even more distinct between persistent infections and permanently HR-HPV negative outcome, with ten covariates predicting the former at the $p = 0.0001$ significance level. These covariates are practically identical with those distinguishing between persistent- and transient HR-HPV infections (9 with $p = 0.0001$) (Table 3).

In the final multivariate model with all significant univariates entered, relatively few covariates are significant in pair-wise comparisons between the three HPV outcomes *(Table 4)*. Number of recent sexual partners and previous CIN history increase the probability of transient HR-HPV infection against permanently HR-HPV negative competing event, while previous Pap screening history is protective. Persistent ASCUS+ Pap during FU and no previous Pap screening history are significantly associated with the persistent outcome (compared with an always negative competing event), whereas multiparity is protective. These three covariates also make the significant distinction between persistent- and transient HR-HPV infections, practically with the same power as obtained when the HR-HPV negative outcome is used as the competing event.

Table 4. — *Covariates associated with transient and persistent HR-HPV outcomes in multivariate competing-risks regression model (with permanently HR-HPV-negative and transient HR-HPV infections as competing events, respectively).*

Competing outcome events	*Adjusted SHR	95% CI		Significance
		Lower Bound	Upper Bound	
Transient HR-HPV Infection (permanently HR-HPV negative as competing event):				
Cohort[1]	omitted			
Age (30-yrs cut-off) (> 30 yrs ref)	1.18	0.95	1.46	0.116
Persistent ASCUS+ Pap smear	1.15	0.81	1.64	0.419
Ever been pregnant (never ref)	0.98	0.76	1.23	0.807
Number of pregnancies[1]	omitted			
Number of live births (0 ref)	0.97	0.86	1.07	0.474
Number of life-time sexual partners[1]	omitted			
Number of recent (< 12 month) sexual partners (0 ref)	1.28	1.03	1.59	**0.020**
Previous Pap smear taken (never ref)	0.72	0.58	0.89	**0.002**
History of previous CIN (no ref)	1.59	1.14	2.22	**0.006**
Persistent HR-HPV Infection (permanently HR-HPV negative as competing event):				
Cohort[1]	omitted			
Age (30-yrs cut-off) (> 30 yrs ref)	1.68	0.75	3.78	0.203
Baseline ASCUS+ Pap smear[1]	omitted			
Incident ASCUS+ Pap smear[1]	omitted			
Persistent ASCUS+ Pap smear (no ref)	8.77	2.67	28.72	**0.0001**
Race (white ref)	0.93	0.38	2.25	0.888
Ever been pregnant (never ref)	0.51	0.12	2.13	0.359
Number of pregnancies (0 ref)	0.52	0.34	0.80	**0.003**
Number of live births[1] (0 ref)	omitted			
Number of life-time sexual partners (1 ref)	1.13	0.71	1.80	0.596
Number of recent (< 12 month) sexual partners	1.03	0.10	10.18	0.975
Mode of contraception	1.01	0.52	1.97	0.963
Previous Pap smear taken	14.03	1.67	117.98	**0.015**
Persistent HR-HPV Infection (transient HR-HPV infection as competing event):				
Cohort[1]	omitted			
Age (30-yrs cut-off) (> 30 yrs ref)	1.54	0.69	3.43	0.284
Baseline ASCUS+ Pap smear[1]	omitted			
Incident ASCUS+ Pap smear[1]	omitted			
Persistent ASCUS+ Pap smear (no ref)	8.05	2.73	23.73	**0.0001**
Race (white ref)	0.92	0.39	2.18	0.861
Ever been pregnant (never ref)	0.56	0.15	2.14	0.404
Number of pregnancies (0 ref)	0.54	0.36	0.82	**0.004**
Number of live births[1] (0 ref)	omitted			
Number of life-time sexual partners (1 ref)	1.09	0.71	1.69	0.676
Number of recent (< 12 month) sexual partners	1.02	0.12	8.32	0.985
Mode of contraception	0.97	0.52	1.81	0.936
Previous Pap smear taken	14.19	1.92	104.51	**0.009**

*Women who remain HC2-negative at repeated testing throughout the FU period; **Both (baseline) prevalent and incident HR-HPV infections that persist for 6+ months; ***Both (baseline) prevalent and incident HR-HPV infections that undergo spontaneous clearance during FU; @Adjusted for all covariates that were significant in univariate model; [1]Omitted from the model because of collinearity; SHR, subhazard ratio; significant covariates are in bold.

Discussion

As evidenced by several cohort studies, the natural history of HPV has several characteristics that, from a statistical point of view, are infrequently encountered in other fields of infectious disease or cancer research [4-11, 18-31, 33]. Despite the fact that multiple-type infections are common, prevalence, incidence, persistence and clearance of HPV can be measured at genotype level in longitudinal settings with repeated sampling [16, 23, 32, 43]. In settings where repeated measures involve the same subject, the results tend to be correlated [33]. In other words, the probability of detecting any given HPV genotype is greater among women who test positive for another genotype, and similarly, women with biopsy-confirmed CIN are more likely to have the disease in the subsequent visit as well, if repeated within a reasonable time frame. Statistical techniques that i) fail to take these correlations into account would be invalid, and ii) methods that do not exploit all the collected data (in a repeated measures setting) would be inefficient [33]. Marginal (e.g., GEE, generalized estimating equation) [16, 23, 32] and mixed-effects models [33] are both capable of handling these issues, showing a greater efficiency as compared with standard logistic regression and Cox models for studying the natural history of HPV infections.

We recently used GEE [32] and Poisson regression for panel data [16, 23] in modeling the covariates associated with genotype-specific HPV persistence, incident HPV and virus clearance in the prospective Finnish Family HPV Cohort. Both techniques would be technically suitable for analysis of the panel data of the present study as well, but because they only accept a binomial (0/1) dependent variable this would necessitate a separate analysis of the multiple comparisons between the three events of interest, which is not feasible. Thus, we ended up in selecting another method for analysing our data, by taking into account the fact that i) the longitudinal data be utilised in full, ii) dependence of the repeated measurements at FU visits be taken into account, and iii) the multiple-endpoint (no-, transient- and persistent HR-HPV infection) variable be treated in a single model. All these prerequisites are met by the competing-risks regression [34,35], here used to model the covariates associated with the competing outcomes of cervical HPV infections.

Based on the method of Fine and Gray (1999), competing-risks regression provides a useful alternative to standard Cox regression for survival data in the presence of competing risks [34]. In contrast to the usual survival analysis measuring time-to-failure (e.g., clearance) as a function of observed covariates, the term competing risk refers to the chance that instead of HPV clearance (i.e., transient infection), one will observe a competing event, e.g. virus persistence or no HPV infection at all [34, 35]. During the observation period, detection of any of these competing events impedes the occurrence of the event of interest. This is basically different from the usual censoring that occurs in conventional survival analysis, i.e., loss to follow-up; while censoring obstructs you from observing the event of interest, a competing event prevents the occurrence of the event of interest. In simple terms, competing-risks regression generates hazard for (failure) events of interest, while simultaneously keeping the subjects who experience competing events still "at risk" so that they can be adequately counted as not a chance of failing [34, 35]. Different from the usual Cox regression models producing HR (hazard ratio), this technique reports exponentiated coefficients known as subhazard ratios (SHR). The correlation within multiple records on the same subject is accounted for by using robust variance estimator, clustered by patient-ID, to treat each observation within a patient as an own predictor and not as a set of overlapping predictors [34, 35].

In classical cohort studies [4-11], the natural history of cervical HPV infections has been shown to be extremely complex, with several distinct outcome patterns observed. The natural history of CIN is closely linked with HPV, albeit less complex (regression, persistence, progression) [1-3]. However, given that HPV is the causative agent of CIN, we should separate the natural history of cause (HPV) and effect (CIN), i.e., to distinguish between viral outcomes and clinical outcomes [4-10]. As a virus, HPV in the cervix can cause incident infection, remain persistent, or undergo spontaneous clearance [12-23]. Because both incident and prevalent HPV infections can either persist or undergo clearance, HPV outcomes can be simplified as either i) persistent, or ii) transient HPV infections. Among baseline HPV-negative women, the third possible outcome is permanent absence of HPV, i.e., no incident event during the longitudinal follow-up. Because these outcomes are mutually exclusive, but a woman still remains "at risk" for the other outcomes, these are competing events, making the data suitable for analysis by competing-risks regression [34, 35].

From the clinical point of view, there is a major difference between these three HPV outcomes. While practically no risk for developing CIN and CC is encountered among women who remain constantly HR-HPV-negative, there is a non-negligible risk for incident CIN2+ among women who experience a transient HR-HPV infection [24-29]. On the other hand, several studies have demonstrated very high relative and absolute risks of CIN2+ ascribable to type-specific persistent HR-HPV infections. Clearly, persistent HR-HPV infections represent a sign of increased risk of CC, and as such, 6-month (6M+) or 12-month (12M+) type-specific persistence of HR-HPV have been proposed as powerful surrogates of progressive disease [31, 42, 44]. As recently shown, however, the power of both 6M+ and 12M+ persistent HR-HPV infections as predictors of incident CIN2+ critically depends on the reference category used in the calculations, i.e., whether transient HPV infections or constantly HPV-negative women [31, 42, 44]. This implicates that the risk of incident CIN2+ is also increased among women with transient infections as compared with constantly HPV-negative women [31, 42]. Another way to look at this is to assess the covariates associated with transient infections on one hand and persistent infections on the other hand, as done in the present study treating these outcomes as competing events.

As clearly shown by the present data, the covariates associated with transient HR-HPV and persistent HR-HPV infections are quite different when HPV-negative outcome is used as the competing event, but very similar indeed, when compared to each other (Table 3). These data provide direct confirmation to the recent discussion, why constantly HPV-negative women should always be used as the reference category while calculating the predictive power of 6M+ and 12M+ HR-HPV persistence as surrogate endpoints of progressive disease [31, 42].

In the present analysis, a large number of covariates were associated with each of the three competing events (Tables 1-4). However, in multivariate competing-risks regression models, only a few remained significant independent predictors of each event (with the two others as competing events). While permanently HR-HPV negative outcome was significantly predicted only by the clearance of ASCUS+ Pap during FU, there were three independent covariates of transient HR-HPV infections: i) number of recent (< 12 months) sexual partners (increase the risk), ii) previous Pap screening history (protective), and history of previous CIN (increased risk). The two most powerful predictors of persistent HR-HPV infections were persistent ASCUS+ Pap during FU (increasing the risk), and previous Pap screening history (protective). Also increased parity was an independent (protective) covariate against persistent HR-HPV infection.

When a similar analysis was repeated for pair-wise comparisons between each three outcomes, relatively few covariates proved to be significant independent predictors of each competing event. Number of recent sexual partners and previous CIN history increase the probability of transient HR-HPV infection against a permanently HR-HPV negative competing event, while previous Pap screening history is protective. Persistent ASCUS+ Pap during FU and no previous Pap screening history are significantly associated with the persistent outcome (compared with an always negative competing event), whereas multiparity is protective. These three covariates also make the significant distinction between persistent- and transient HR-HPV infections, practically with the same power as obtained with the HR-HPV negative competing event.

These results might have important practical implications, while providing the potential means to address the burning questions arising after a single HPV test, irrespective of whether HPV-positive or HPV-negative. In the former case, the patient is interested in the eventual outcomes and their predictability. In the latter case, the issues are related to the protective effect of a single negative HPV test, i.e., probability of remaining HPV-negative also in the foreseeable future. If accurately predicted, substantial savings can be achieved while refraining from repeat HPV testing of these HPV-negative women who are likely to remain HPV-negative also in the future. In HPV-positive cases, on the other hand, an accurate distinction of transient infections from HR-HPV persistence would avoid unnecessary treatments of infections that are likely to resolve, but instead help in focusing the efforts of monitoring the women at high risk for incident CIN2+ [24-29, 31, 42].

Taken together, the present analysis of a sub-cohort of 1,865 women in the combined NIS-LAMS cohort used competing-risks regression models to disclose significant covariates associated with three main outcomes (persistent-, transient-, or no HR-HPV infection) of cervical HPV infections, treated as competing events. Covariates associated most significantly with each of the three competing events were distinct enough to enable designing a risk-profile for each outcome. In the next step, the performance of these risk-profiles in predicting the longitudinal outcomes of cervical HPV infections will be tested in this cohort.

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Address reprint requests to:
K. SYRJÄNEN, M.D., Ph.D., FIAC
Department of Oncology and Radiotherapy
Turku University Hospital
Savitehtaankatu 1, FIN-20521 Turku (Finland)
e-mail: kari.syrjanen@tyks.fi