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How to cite

CLIFFORD, Gary M et al. Immunodeficiency and the risk of cervical intraepithelial neoplasia 2/3 and cervical cancer: A nested case-control study in the Swiss HIV cohort study. In: International journal of cancer, 2016, vol. 138, n° 7, p. 1732–1740. doi: 10.1002/ijc.29913

This publication URL: https://archive-ouverte.unige.ch/unige:89121

Publication DOI: 10.1002/ijc.29913

IJC
International Journal of Cancer

Immunodeficiency and the risk of cervical intraepithelial neoplasia 2/3 and cervical cancer: A nested case-control study in the Swiss HIV cohort study

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HIV-infected women are at increased risk of cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (ICC), but it has been difficult to disentangle the influences of heavy exposure to HPV infection, inadequate screening and immunodeficiency. A case-control study including 364 CIN2/3 and 20 ICC cases matched to 1,147 controls was nested in the Swiss HIV Cohort Study (1985–2013). CIN2/3 risk was significantly associated with low CD4+ cell counts, whether measured as nadir [odds ratio (OR) per 100-cell/μL decrease = 1.15, 95% CI: 1.08, 1.22], or at CIN2/3 diagnosis (1.10, 95% CI: 1.04, 1.16). An association was evident even for nadir CD4+ 200–349 versus ≥350 cells/μL (OR = 1.57, 95% CI: 1.09, 2.25). After adjustment for nadir CD4+, a protective effect of >2-year cART use was seen against CIN2/3 (OR versus never cART use = 0.64, 95% CI: 0.42, 0.98). Despite low study power, similar associations were seen for ICC, notably with nadir CD4+ (OR for 50 *vs.* >350 cells/μL= 11.10, 95% CI: 1.24, 100). HPV16-L1 antibodies were significantly associated with CIN2/3, but HPV16-E6 antibodies were nearly exclusively detected in ICC. In conclusion, worsening immunodeficiency, even at only moderately decreased CD4+ cell counts, is a significant risk factor for CIN2/3 and cervical cancer.

Key words: cervical cancer, cervical neoplasia, HIV, human papillomavirus, immunodeficiency

Abbreviations: AIDS: acquired immunodeficiency syndrome; cART: combined antiretroviral therapy; CI: confidence interval; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; ICC: invasive cervical cancer; OR: odds ratio; Pap: Papanicolaou; SHCS: Swiss HIV Cohort Study; WHIV: women infected with human immunodeficiency virus

[†]The members of the Swiss HIV Cohort Study are listed in the Acknowledgements section.

Conflict of interest: BB has received consultation and speaker's fees and occasional travel funds to scientific meetings from AbbVie, ViiV, BMS, Gilead and Janssen. The other authors have no conflict of interest to declare.

Grant sponsor: Swiss HIV Cohort Study (SHCS-project 676); **Grant sponsor:** Swiss National Science Foundation; **Grant number:** 148522; **Grant sponsor:** OncoSuisse; **Grant numbers:** ICP OCS 01355–03-2003 and KFS-02478–08-2009.
DOI: 10.1002/ijc.29913
History: Received 11 Aug 2015; Accepted 19 Oct 2015; Online 4 Nov 2015

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What's new?

Women infected with human immunodeficiency virus (HIV) suffer from precancerous cervical lesions and severe invasive cervical cancer (ICC) at rates that far exceed those of their HIV-free counterparts. Our case-control study nested in the Swiss HIV Cohort Study demonstrated the role of immunodeficiency and notably CD4+ cell nadir in the onset of CIN2/3 and ICC. Even moderate declines in CD4+ cell counts substantially increased the risk of cervical intraepithelial neoplasia grade 2/3. The findings emphasize a key role for early HIV detection and combined antiretroviral therapy initiation in cervical cancer prevention for HIV-infected women.

There is a substantial excess of invasive cervical cancer (ICC) among women infected with HIV (WHIV).[1] This excess has tended to be largest in settings where WHIV have access to combined antiretroviral therapy (cART), but are not well reached by cervical screening.[2–4] Studies have also shown a consistent excess of cervical intraepithelial neoplasia grade 2/3 (CIN2/3) in WHIV.[5,6]

Sexual behaviors that favor acquisition of HIV are the same that favor the acquisition of high-risk human papillomavirus (HPV), which leads to a higher prevalence of HPV in WHIV.[7] In addition, however, HIV-related immunodeficiency is known to unfavorably influence HPV natural history. CD4+ cell count is negatively associated with the prevalence,[8–11] persistence[12] and cumulative incidence[13] of HPV infection, as well as with cytological abnormalities[11] and CIN2/3[6,14,15] among WHIV.

The effects of HIV-related immunodeficiency on ICC have been more difficult to establish. First, ICC has been classified as an acquired immunodeficiency syndrome (AIDS)-defining cancer since 1993, which created problems for early linkage studies between people with AIDS and cancer registries. Many prevalent cervical cancers diagnosed at AIDS onset had to be excluded from estimations of relative risk.[16] Second, screening can substantially curb the ICC burden.[17] Only in recent years are large studies of WHIV revealing that CD4+ cell count is indeed negatively related to ICC risk.[18–20]

The dose response relationship between the level of immunodeficiency and the risk of CIN2/3 and ICC remains unclear, however, as does the effect of cART-induced improvements in immunity on the evolution of cervical neoplasia. Thus, we undertook a case-control study nested within the Swiss HIV Cohort Study (SHCS), a nationwide study of HIV-positive persons, to characterize the influence of immunodeficiency and cART use on the development of CIN2/3 and ICC among WHIV.

Material and Methods

The Swiss HIV cohort study

The SHCS is a nationwide prospective cohort that has been enrolling HIV-infected persons aged 16 years or older since 1988 from all five Swiss University hospitals (Basel, Bern, Geneva, Lausanne and Zurich) and two cantonal hospitals (St Gallen and Ticino).[21] Since 1995, interested private physicians have also been enrolling patients. Twenty-eight percent of participants in the SHCS are women. The database used for the current study included a total of 5,150 female SHCS participants contributing ~35,000 person years of active follow-up up to October 2013. Detailed information on all AIDS-related diseases, CD4+ cell count and HIV-related treatments were collected at enrollment and at each six-month follow-up visit. Questions on smoking history were asked to SHCS participants at all enrollment and follow-up visits after April 2000.

History of Papanicolaou (Pap) smear has been self-reported by SHCS participants at all enrollment and follow-up SHCS visits since April 2001. Pap smear history was censored at the SHCS visit preceding the reference date (see definition below), which should exclude the smear that led to diagnosis of CIN2/3 or ICC.

Written informed consent was obtained from all SHCS participants. The present study was approved by the local ethical committees of the seven SHCS sites and of the International Agency for Research on Cancer.

Ascertainment of incident cases

Cases of CIN2/3 and of ICC are routinely recorded in the SHCS. Additional cases were identified through record linkage with eight cantonal cancer registries.[22,23] Prevalent CIN2/3 and ICC cases were defined as women diagnosed before, or within one month of enrollment into the SHCS and were excluded from analyses.

Selection of controls

Three control women never diagnosed with CIN2/3 or ICC were matched to each CIN2/3 or ICC case using incidence density sampling.[24] Controls had at least the same length of follow-up as the matched case and could serve as controls for only one case. Matching criteria were: (*i*) SHCS centre; (*ii*) HIV-transmission category (injecting drug users, heterosexual/other); (*iii*) age at enrollment in SHCS (as close as possible, up to a maximum of nine years difference); (*iv*) calendar year of enrollment in SHCS (as close as possible, up to a maximum of nine years difference). For each case a list of possible controls was drawn up and three controls were selected at random.

Definitions

For each case-control pair, the reference date was defined as the date corresponding to the same length of follow-up as the matched case had at the time of the diagnosis of CIN2/3 or ICC (thus cases and controls were also matched for age and year of reference date). The nadir CD4+ cell count was defined as the lowest ever reported CD4+ cell count prior to the reference date. cART use was defined as a combination of at least three antiretroviral drugs, including a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor or three nucleosides, including abacavir. Only persons who had used cART for more than one month prior to the reference date were classified as ever users and cART users were additionally stratified by duration of use from date of cART initiation to reference date ($\leq$2 *vs.* >2 years). This duration may include periods of interruptions, but these occur in only a small fraction of SHCS participants on cART (5–10%).[25]

History of AIDS was defined as a history of any AIDS-defining illness prior to the reference date. Of note, for ICC and corresponding controls, AIDS-defining events were counted only if diagnosed at least 3 months before the reference date, to avoid counting ICC that was the first AIDS-defining illness.

We considered CIN2/3 and ICC to be different biological entities and chose not to combine them in any analyses.

HPV serology

For a sub-set of CIN2/3 and ICC cases with available serum samples (79 and 13, respectively), as well as 248 corresponding controls, HPV antibodies were tested in the serum samples taken closest in time prior to the reference date. HPV antibodies were tested at the German Cancer Research Center (DKFZ) in Heidelberg, Germany, using multiplex bead-based technology (xMAP, Luminex Corporation, Austin, Texas)[26] including antigens for L1 and E6 proteins of HPV16. Antigens were categorized as antibody positive or negative by applying previously defined antigen-specific cut-off values.[27]

Statistical analysis

Odds ratios (OR) and corresponding 95% confidence intervals (CI) for possible risk factors were computed separately for CIN2/3 and ICC by univariate conditional logistic regression conditioned on matching variables. For CD4+ cell count measures, OR were also calculated per 100 cells/μl decrease considering CD4+ cell count as a continuous variable. The OR for cART use adjusted for nadir CD4+ cell count was additionally computed. In analyses stratified by ever versus never cART use, however, OR for nadir CD4+ cell count were computed by unconditional logistic regression with adjustment for matching variables (SHCS centre; HIV-transmission category; age at enrollment; year at enrollment), due to the need to break case:control pairs (cART use was not a matching criteria). Missing data were considered as a separate category in the above models. For smoking and Pap smear history that were unavailable prior to April 2000 and April 2001, respectively, conditioning on year at reference date meant that most missing data clustered in the same case:control pairs, which were effectively dropped from the estimates for these two variables). χ^2_1 for trend was calculated for the categories listed in the tables. p values of all statistical tests were two-sided.

Median CD4+ cell counts were calculated separately for never and ever cART users in yearly periods prior to the reference date, restricted to cases and controls who (*i*) were under active follow-up in that period and (*ii*) had a valid CD4+ cell count, in each yearly period. If more than one measurement was available during any one time period, that closest to the reference date was used. Matching was not retained in the presentation of median CD4+ cell counts.

Results

A total of 508 CIN2/3 and 40 ICC cases were identified in SHCS participants, of whom 437 and 32 were identified from the SHCS database and 71 and 8 additional cases were identified through record linkage with eight population-based Swiss Cantonal Cancer Registries.[22,23] One hundred and forty CIN2/3 and 16 ICC occurring before (or within one month of) SHCS enrollment, as well as four CIN2/3 and four ICC diagnosed more than six months after the last SHCS follow-up date, were excluded, leaving 364 and 20 eligible incident cases occurring during active SHCS follow-up, respectively.

Table 1 shows the distribution of the CIN2/3 and ICC cases, as well as their respective controls, by matching variables. For five CIN2/3 cases, only two valid controls were available. A majority of both CIN2/3 and ICC cases were diagnosed after the introduction of cART in 1996 (72 and 65% respectively), and had been under active follow-up in the SHCS for less than five years prior to diagnosis (66 and 60%, respectively). However, ICC cases (mean age 34.3 years) were older than CIN2/3 cases (29.7).

There were no significant associations with history of Pap smear, neither for CIN2/3 nor ICC. Nevertheless, findings did suggest frequent lack of screening among ICC cases (37.5%; Table 2). There was no association of smoking history with CIN2/3 and, although 75% of ICC cases were current or former smokers compared to 51% of controls, this difference did not reach statistical significance. Likewise, no associations with a history of AIDS were observed for either CIN2/3 or ICC (Table 2).

Associations of CIN2/3 and ICC with various markers of immunodeficiency are also shown in Table 2. CIN2/3 (OR per 100 cell/μL decrease = 1.10, 95% CI: 1.04, 1.16) and ICC (1.16, 95% CI: 0.89, 1.51) were similarly associated with low CD4+ cell count at reference date, although the ICC association did not reach statistical significance. CIN2/3 was also negatively associated with CD4+/CD8+ ratio (OR for <0.25 *vs* >0.50 = 1.67, 95% CI: 1.19, 2.35). No significant trend in association with CIN2/3 or ICC was observed for HIV viral load (Table 2), or CD8+ cell count (data not shown).

Table 1. Distribution of 364 CIN 2/3 cases, 20 invasive cervical cancer cases and 1,147 control subjects according to matching variables

	CIN2/3				ICC			
	Cases		Controls		Cases		Controls	
	N	(%)	*N*	(%)	*N*	(%)	*N*	(%)
Overall	364		1087		20		60	
Center								
Basel	25	(6.9)	75	(6.9)	2	(10.0)	6	(10.0)
Bern	81	(22.3)	241	(22.2)	3	(15.0)	9	(15.0)
Geneva	54	(14.8)	162	(14.9)	1	(5.0)	3	(5.0)
St Gallen	45	(12.4)	134	(12.3)	–	–	–	–
Ticino	22	(6.0)	64	(5.9)	–	–	–	–
Vaud	44	(12.1)	132	(12.1)	5	(25.0)	15	(25.0)
Zurich	93	(25.5)	279	(25.7)	9	(45.0)	27	(45.0)
HIV-transmission category								
IDU	139	(38.2)	413	(38.0)	9	(45.0)	27	(45.0)
Het/Other	225	(61.8)	674	(62.0)	11	(55.0)	33	(55.0)
Age at reference date (years)[1]								
<30	91	(25.0)	268	(24.7)	3	(15.0)	10	(16.7)
30–34	119	(32.7)	328	(30.2)	6	(30.0)	13	(21.7)
35–39	87	(23.9)	276	(25.4)	2	(10.0)	16	(26.7)
≥40	67	(18.4)	215	(19.8)	9	(45.0)	21	(35.0)
Calendar period at reference date[1]								
1985–1992	22	(6.0)	86	(7.9)	3	(15.0)	11	(18.3)
1993–1996	80	(22.0)	201	(18.5)	4	(20.0)	5	(8.3)
1997–2001	120	(33.0)	326	(30.0)	4	(20.0)	17	(28.3)
2002–2006	76	(20.9)	293	(27.0)	5	(25.0)	22	(36.7)
2007–2013	66	(18.1)	181	(16.7)	4	(20.0)	5	(8.3)
Duration of follow-up before reference date (years)[1]								
0–1	146	(40.1)	437	(40.2)	8	(40.0)	24	(40.0)
2–4	93	(25.5)	280	(25.8)	4	(20.0)	12	(20.0)
5–9	88	(24.2)	266	(24.5)	2	(10.0)	6	(10.0)
≥10	37	(10.2)	104	(9.6)	6	(30.0)	18	(30.0)

Abbreviations: CIN: cervical intraepithelial neoplasia; Het: heterosexual; ICC: invasive cervical cancer; IDU: intravenous drug users.
[1]Reference date for cases = date of diagnosis of ICC or CIN2/3 and for controls = date after a similar length of follow-up in the Swiss HIV Cohort Study as the corresponding matched case.

CIN2/3 was significantly associated with a low nadir CD4+ cell count (OR per 100 cells/μL decrease = 1.15, 95% CI: 1.08, 1.22), even at moderate levels of immunosuppression (OR for 200–349 *vs.* ≥350 cells/μL = 1.57, 95% CI: 1.09, 2.25). For ICC, an equally strong trend as for CIN2/3 was found (OR per 100 cells/μL decrease = 1.19, 95% CI: 0.88, 1.60), but only the comparison of nadir CD4+ cell count of <50 versus ≥500 cells/μL reached statistical significance (OR = 11.10, 95% CI: 1.24, 100).

About half of CIN2/3 and ICC cases had started cART before the reference date (Table 3), but there were no significant differences in cART use between cases and controls for either disease. Upon stratification by duration of cART use, however, women with ≤2 years use showed elevated CIN2/3 risks compared with never users, whilst those with >2 years use showed nonsignificantly reduced risks. After additional adjustment for nadir CD4+ cell count, the OR for >2-years cART use was associated with a significant reduction in CIN2/3 risk (OR = 0.64, 95% CI: 0.42, 0.98). A similar association, albeit nonsignificant, was seen for ICC risk (*e.g.*, OR for ≥2 years *vs.* never = 0.34, 95% CI: 0.05, 2.26; Table 3).

Table 4 shows associations of nadir CD4+ cell count with CIN2/3 and ICC, stratified by cART use (never/ever). Nadir CD4+ cell count was significantly associated with CIN2/3

Table 2. ORs for CIN 2/3 and invasive cervical cancer by selected characteristics at reference date[1]

	CIN2/3					ICC				
	Cases		Controls		OR[2] (95% CI)	Cases		Controls		OR[2] (95% CI)
	N	(%)	*N*	(%)		*N*	%	*N*	%	
Overall	364		1087			20		60		
History of Pap smear[3]										
No	40	(29.2)	129	(28.1)	1	3	(37.5)	5	(20.8)	1
Yes	97	(70.8)	330	(71.9)	0.90 (0.57, 1.41)	5	(62.5)	19	(79.2)	0.39 (0.06, 2.45)
Unknown	227		628			12		36		
Smoking Status[4]										
Never	89	(43.4)	289	(45.6)	1	3	(25.0)	18	(48.6)	1
Former	17	(8.3)	59	(9.3)	0.93 (0.51, 1.70)	3	(25.0)	6	(16.2)	3.73 (0.75, 18.6)
Current	99	(48.3)	286	(45.1)	1.13 (0.78, 1.64)	6	(50.0)	13	(35.1)	
Unknown	159		453			8		23		
History of AIDS[5]										
No	280	(76.9)	851	(78.3)	1	14	(70.0)	45	(75.0)	1
Yes	84	(23.1)	236	(21.7)	1.08 (0.81, 1.45)	6	(30.0)	15	(25.0)	1.36 (0.39, 4.69)
Unknown	227		628			12		36		
CD4+ cell count (cells/μL)										
≥500	93	(27.4)	350	(35.7)	0.89 (0.62, 1.26)	3	(17.6)	20	(37.0)	0.95 (0.14, 6.60)
350–499	70	(20.6)	238	(24.3)	1	2	(11.8)	10	(18.5)	1
200–349	87	(25.7)	202	(20.6)	1.51 (1.04, 2.18)	8	(47.1)	15	(27.8)	3.49 (0.58, 21.1)
<200	89	(26.3)	191	(19.5)	1.72 (1.16, 2.54)	4	(23.5)	9	(16.7)	3.04 (0.38, 24.3)
Unknown	25		106			3		6		
χ^2_1 trend (*p*)					*< 0.001*					*0.062*
Per 100 μL decrease					1.10 (1.04, 1.16)					1.16 (0.89, 1.51)
CD4+/CD8+ ratio										
>0.50	140	(41.5)	502	(51.4)	1	6	(35.3)	23	(43.4)	1
0.25–0.49	111	(32.9)	279	(28.6)	1.47 (1.09, 1.97)	6	(35.3)	23	(43.4)	1.38 (0.46, 4.16)
<0.25	86	(25.5)	195	(20.0)	1.67 (1.19, 2.35)	5	(29.4)	7	(13.2)	
Unknown	27		111			3		7		
χ^2_1 *trend (p)*					*< 0.001*					
HIV Viral load, copies/mL										
<500	175	(64.8)	472	(59.3)	1	8	(66.7)	22	(51.2)	1
500–9,999	37	(13.7)	168	(21.1)	0.59 (0.39, 0.88)	1	(8.33)	9	(20.9)	0.53 (0.12, 2.33)
≥10,000	58	(21.5)	156	(19.6)	1.00 (0.70, 1.44)	3	(25.0)	12	(27.9)	
Unknown	94		291			8		17		
χ^2_1 *trend (p)*					*0.26*					
Nadir CD4+ cell count, cells/μL										
≥350	68	(18.8)	323	(29.9)	1	2	(10.0)	16	(27.1)	1
200–349	88	(24.4)	280	(25.9)	1.57 (1.09, 2.25)	4	(20.0)	15	(25.4)	2.94 (0.44, 19.9)
50–199	135	(37.4)	314	(29.0)	2.20 (1.55, 3.11)	9	(45.0)	21	(35.6)	4.45 (0.81, 24.4)
<50	70	(19.4)	165	(15.2)	2.20 (1.47, 3.30)	5	(25.0)	7	(11.9)	11.10 (1.24, 100)
Unknown	3		5			0		1		
χ^2_1 *trend (p)*					*<0.001*					*0.019*
Per 100 μL decrease					1.15 (1.08, 1.22)					1.19 (0.88, 1.60)

Abbreviations: CI: confidence interval; CIN: cervical intraepithelial neoplasia; ICC: invasive cervical cancer; OR: odds ratio.
[1]Reference date for cases = date of diagnosis of ICC/CIN2/3 and for controls = date after a similar length of follow-up in the SHCS as the matched case; [2]Conditioned on matching variables; [3]Available only after April 2001; [4]Available only after April 2000; [5]For ICC and corresponding controls, history of AIDS only if ≥3 months before ICC (see methods).

Table 3. ORs for CIN 2/3 and invasive cervical cancer by cART use before and after adjustment for nadir CD4 cell count

History of cART use	Cases		Controls		OR[1] (95% CI)	Adjusted OR[2] (95% CI)
	N	(%)	*N*	(%)		
CIN2/3	364		1087			
Never	160	(44.0)	513	(47.2)	1	1
Ever	204	(56.0)	574	(52.8)	1.24 (0.90, 1.70)	0.96 (0.68, 1.35)
≤2years	103	(28.3)	231	(21.3)	1.59 (1.12, 2.26)	1.25 (0.86, 1.81)
>2years	101	(27.7)	343	(31.5)	0.87 (0.59, 1.29)	0.64 (0.42, 0.98)
ICC	20		60			
Never	10	(50.0)	32	(53.3)	1	1
Ever	10	(50.0)	28	(46.7)	1.19 (0.37, 3.82)	0.62 (0.15, 2.54)
≤2years	4	(20.0)	8	(13.3)	1.60 (0.40, 6.30)	0.97 (0.20, 4.66)
>2years	6	(30.0)	20	(33.3)	0.78 (0.16, 3.86)	0.34 (0.05, 2.26)

Abbreviations: cART: combined antiretroviral therapy; CI: confidence interval; CIN: cervical intraepithelial neoplasia; ICC: invasive cervical cancer; OR: odds ratio.
[1]Conditioned upon matching variables.
[2]Conditioned upon matching variables and adjusted for nadir CD4 categories in Table 2.

risk both among ever users (OR per 100 cell/μL decrease = 1.12, 95% CI: 1.03, 1.21) and never users (1.17, 95% CI: 1.05, 1.30). Nadir CD4+ cell count was strongly associated with ICC risk in never users (OR for <50 *vs.* ≥350 cells/μL = 12.90, 95% CI: 0.38, 436, p for trend = 0.004), but the corresponding associations did not reach statistical significance in ever users (OR for <50 *vs.* ≥350 cells/μL = 6.41, 95% CI: 0.43, 95.1, p for trend = 0.26).

Figure 1 shows median CD4+ cell counts in yearly periods prior to the reference date in CIN2/3 cases and controls, separately for never and ever cART users. As expected, mean CD4+ cell counts among both cases and controls declined over time among never cART users (Fig. 1*a*) but were stable in ever cART users (Fig. 1*b*). Median CD4+ cell count was lower in cases compared with that in controls at most time points prior to the reference date, and the size of this difference did not appear to vary by time period, neither for never or ever users. Indeed, the association between CIN2/3 risk and more historical measures of CD4+ count was similar to that for CD4+ cell count at reference date (*e.g.*, matched OR per 100 cells/μL decrease for CD4+ cell count 3–4 years prior to reference date = 1.15, 95% CI: 1.05–1.25, data not shown). Numbers were too few to allow a similar analysis for ICC.

Seropositivity for anti-HPV16E6 was found in 2 out of 13 ICC cases (15%) compared with 0 out of 37 controls. Three out of 79 CIN2/3 cases and 6 out of 211 corresponding controls were seropositive for anti-HPV16E6. Seroprevalence of anti-HPV16L1 was significantly higher in CIN2/3 cases (40%) than controls (26%; OR = 1.93, 95% CI: 1.09, 3.40; Fig. 2).

Discussion

Our carefully matched case-control study within the SHCS was able to confirm the role of immunodeficiency in the etiology of CIN2/3[14,15] and ICC[18–20] among WHIV. Furthermore, we showed that increases in CIN2/3 and ICC risk are already evident even at moderate levels of immunosuppression (200–349 cells/μL), similar to our recent findings in the SHCS for anal cancer, which is also HPV-related.[28] Low CD4+ cell counts at diagnosis were significantly associated with risk of CIN2/3, but nadir CD4+ cell count appeared a more discriminant CD4+ measure of both CIN2/3 and ICC.

We were able to demonstrate a protective effect of cART use on the risk of CIN2/3, but exclusively if the use had lasted 2 years or more. Whereas early studies failed to show a significant effect of cART use on HPV-related outcomes, others have since reported significant decreases in prevalence of HPV infection,[15,29] incidence of cytological lesions,[30] or carcinoma *in situ*/ICC[4] in medium/long-term users. In our study, the protective effect of cART use on CIN2/3 was strengthened by adjusting for nadir CD4+ cell count, which partially adjusts for the negative confounding of CD4+ cell count as an indication to treat. Indeed, cART tends to be initiated only when CD4+ cell count falls below a certain level, so that non-cART users had higher average nadir CD4+ cell counts than cART users. In our present study, mean nadir CD4+ cell count in cART users was 163 in CIN2/3 and 207 in corresponding controls, versus 293 and 363, respectively, among non-cART users. Previous findings of lack of influence of cART may thus be due to lack of accounting for duration of treatment and/or CD4+ cell count at cART initiation.

As more than half of cases and controls had never used cART at the reference date, we were able to show the effect of nadir CD4+ cell count on CIN2/3 both in ever and never users of cART.[14,15] These findings suggest that cART use can only partially eliminate the negative influence of a low nadir on CIN2/3, which differs from other AIDS-defining malignancies like immunoblastic non-Hodgkin lymphoma[31] and Kaposi sarcoma[32] in which cART can rapidly lower cancer risk. Hence, the benefits of

Table 4. ORs for CIN 2/3 and invasive cervical cancer by nadir CD4+ count, separately among never and ever cART users

Nadir CD4+ cell count (cells/μL)	Never cART users					Ever cART users				
	Cases		Controls			Cases		Controls		
	N	(%)	*N*	(%)	OR[1] (95% CI)	*N*	%	*N*	%	OR[1] (95% CI)
CIN2/3	160		513			204		574		
≥350	51	(32.5)	229	(45.1)	1	17	(8.3)	94	(16.4)	1
200–349	43	(27.4)	126	(24.8)	1.53 (0.96, 2.43)	45	(22.1)	154	(26.8)	1.67 (0.90, 3.01)
50–199	42	(26.8)	99	(19.5)	1.85 (1.13, 3.05)	93	(45.6)	215	(37.5)	2.51 (1.40, 4.48)
<50	21	(13.4)	54	(10.6)	1.78 (0.97, 3.26)	49	(24.0)	111	(19.3)	2.58 (1.37, 4.84)
Unknown	3		5			0		0		
χ^2_1 *trend (p)*					*0.013*					*<0.001*
Per 100/μL decrease					1.12 (1.03,1.21)					1.17 (1.05, 1.30)
ICC	10		32			10		28		
≥350	1	(10.0)	14	(45.2)	1	1	(10.0)	2	(7.1)	1
200–349	4	(40.0)	11	(35.5)		0	(0.0)	4	(14.3)	
50–199	4	(40.0)	5	(16.1)	4.10 (0.60, 28.1)	5	(50.0)	16	(57.1)	2.55 (0.20, 31.8)
<50	1	(10.0)	1	(3.2)	12.90 (0.38, 436)	4	(40.0)	6	(21.4)	6.41 (0.43, 95.1)
Unknown	0		1			0		0		
χ^2_1 *trend (p)*					*0.004*					*0.26*
Per 100/μL decrease					1.65 (1.00, 2.72)					1.05 (0.58, 1.88)

Abbreviations: cART: combined antiretroviral therapy; CI: confidence interval; CIN: cervical intraepithelial neoplasia; ICC: invasive cervical cancer; OR: odds ratio.
[1]Adjusted for matching variables (see methods).

cART on ICC may be limited by the nonreversibility of certain preinvasive stages.

Cervical cancer screening is a prerequisite for diagnosis of CIN2/3, and its early detection and treatment has undoubtedly curbed incidence rates of ICC in the SHCS. Even in the SHCS, however, screening has been suboptimal, with 20–30% of WHIV in the control groups reporting no history of Pap smear screening. Indeed, among the eight ICC cases diagnosed after 2001 with information on past screening, five had no history of Pap smear. Two ICC cases reported abnormal Pap smears and one case a normal Pap smear in the two years prior to ICC diagnosis. Thus, the excess risk of ICC seen during the pre-cART period in the SHCS in comparison to the general population[22] is likely to be a combination of immunodeficiency and inadequate screening and/or treatment of precancerous lesions.

Our limited data on anti-HPV16 L1 seroprevalence demonstrates the high burden of HPV in SHCS women. Thirty percent of women in the control group were seropositive for anti-HPV16 L1, which compares to only 10% among mixed-sex controls from a large European-wide population-based study using the same serological assay.[33] Furthermore, whilst anti-HPV16 L1 is considered the best marker of cumulative exposure to HPV16 infection, the reported seroprevalence is certainly an under-estimate as a substantial fraction of HPV infections are known not to elicit L1 seroconversion.[34]

Antibodies to HPV16 E6 were seen in ICC, but were almost absent, not only in controls, but also in CIN2/3, confirming them as a marker specific to late stages of HPV malignant transformation in the anogenital tract.[33] Nevertheless, only 15% of ICC cases were seropositive for antibodies against HPV16 E6, which compares to 32% seen in serum samples taken close in time to ICC in the HIV-negative population.[35]

An association, albeit nonsignificant, between smoking and ICC risk in WHIV is consistent with the sexual behavior-adjusted findings from a large pooled analysis of ICC in HIV-uninfected women[36] and with the significant association seen for anal cancer in the SHCS[28] and CIN2/3 in WHIV in the U.S.[6]

The SHCS has many strengths, including the duration (pre-dating the introduction of cART) and regularity of follow-up and medication, as well as the comprehensiveness of clinical and laboratory information. Approximately half of all persons with HIV in Switzerland have been enrolled in the SHCS, and females and non-cART users are well represented. An additional strength was the supplement of CIN2/3 and ICC diagnoses obtained through linkage with cancer registries. However, the SHCS was not designed to evaluate the natural history of HPV infection and related diseases, with gynecological consultations occurring largely outside the framework of the study. Hence, Pap smear history was based on self-report, being added in 2001 only, without any information on cytological diagnoses. In addition, the SHCS does not collect the ideal type of sample for HPV testing (*i.e.*, cervical cells and/or biopsies). This meant that it was not possible to review histological diagnoses in order to confirm ICC or to separate CIN 2 from the truer cancer precursor,

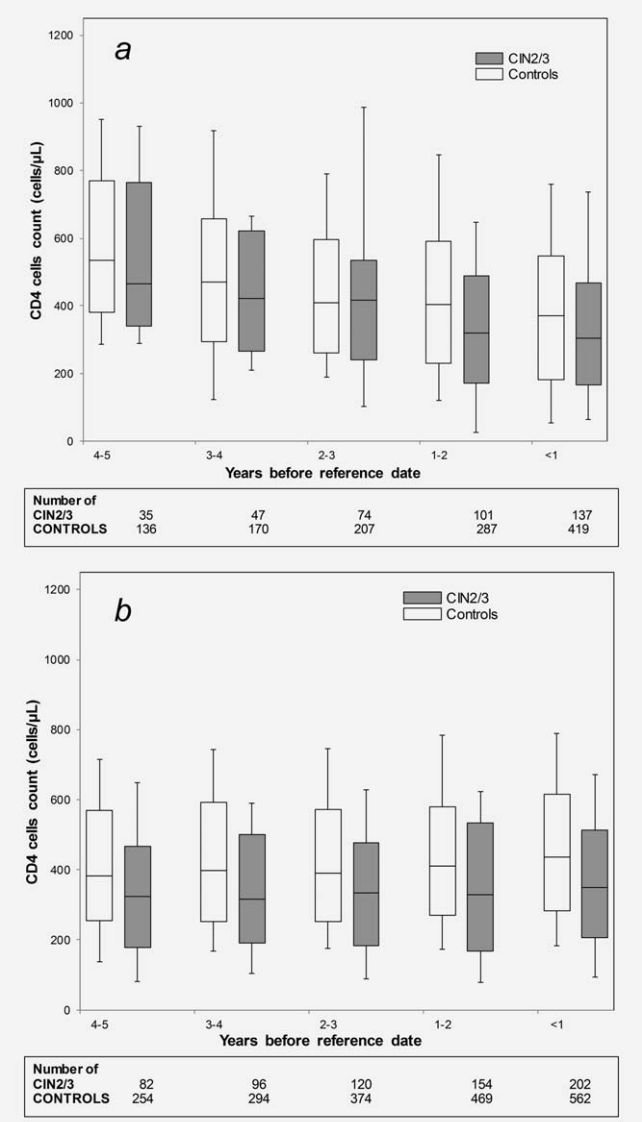

Figure 1. Box plots of CD4+ cell counts at yearly intervals prior to reference date, among CIN 2/3 and controls, stratified by (a) never cART users and (b) ever cART users. Abbreviations: CIN2/3 = cervical intraepithelial grade 2/3; cART = combined antiretroviral therapy.

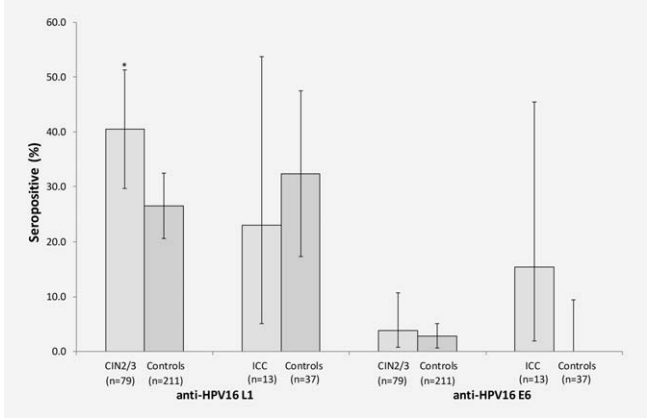

Figure 2. Seropositivity for HPV16 antibodies among CIN 2/3, invasive cervical cancer and corresponding controls. *Significantly different from corresponding controls in conditional logistic regression model. Abbreviations: HPV: human papillomavirus; CIN2/3: cervical intraepithelial grade 2/3; ICC: invasive cervical cancer.

CIN3.[37] Furthermore, the completeness of recording of CIN2/3 in the SHCS is known to differ over time and between cantons. This is one reason that a nested case:control design with careful matching is probably more appropriate than an incidence rate approach. Although ICC cases were few and gave us limited statistical power to achieve significant associations, previous data are very scant. Indeed, the historically suboptimal level of cervical screening and treatment in the SHCS meant that the observed rate of ICC was higher than in well-screened cohort studies of WHIV.[6]

In conclusion, our data suggest that worsening immunodeficiency, even at only moderately decreased CD4+ cell counts (200–349 CD4+ cells/μL), is a significant risk factor for CIN2/3 and cervical cancer. Thus, the earliness at which HIV infection is detected and cART is subsequently initiated is, in combination with HPV vaccination and cervical screening, key to the prevention of cervical cancer in WHIV.

Acknowledgements

The authors thank Dr Kristina Michael for support in the serological analyses and Dr Luigino Dal Maso and Dr Jerry Polesel for data linkage between the SHCS and cancer registries. The members of the Swiss HIV Cohort Study are Aubert V, Battegay M, Bernasconi E, Böni J, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (Deputy of "Positive Council"), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos R, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Metzner K, Müller N, Nadal D, Nicca D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rickenbach M (Head of Data Center), Rudin C (Chairman of the Mother & Child Substudy), Schöni-Affolter F, Schmid P, Schüpbach J, Speck R, Tarr P, Trkola A, Vernazza P, Weber R, Yerly S.

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