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Peripheral leukocyte counts and risk of chlamydia trachomatis (ct), human papilloma viruses (HPV) and cervical disease: a cross-sectional study in a cohort of young women

Abstract

Background: Differential white blood cell (WBC) counts are routinely used as a marker of overall health status as abnormal values can indicate infection, cancer, and other diseases. Recently, differences in WBC within normal ranges have been associated with health outcomes. The human Papilloma virus (HPV) 16/18 vaccine trial in Guanacaste, Costa Rica enrolled 5,711 sexually active healthy women between the ages of 18 and 25 at which time all participants had blood collected for a complete blood count (CBC) as part of a routine clinical assessment.

Methods: Using polytomous regression we investigated the associations between circulating leukocyte measures within CBC (granulocytes, lymphocytes, monocytes, total WBC) and *chlamydia trachomatis* (CT), HPV and HPV-associated low or high squamous intraepithelial lesions (LSIL or HSIL) present at the time of blood collection. Odds ratios (OR) and 95% confidence intervals (95% CI) are presented.

Results: Higher lymphocyte counts were associated with a decreased risk of HSIL in women with carcinogenic HPV infection (OR)Lymphocyte high vs. low tertile: 0.62; 95% CI (0.43, 0.88); P-trend=0.02). No significant associations were observed between leukocyte counts and CT status.

Conclusion: The associations we observed between high lymphocyte counts and a decreased prevalence of carcinogenic HPV positive HSIL were significant. Additional studies investigating the role of lymphocyte counts as an indirect risk factor for HPV persistence are needed. In the future, large studies linking peripheral and local leukocyte counts to phenotype and function may identify subsets which are protective from carcinogenic HPV positive HSIL.

Keywords: human papillomavirus, complete blood count, leukocytes, cervical disease

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Abbreviations: ASCUS, atypical cells of undetermined significance; BMI, body mass index; CT, chlamydia trachomatis; CBC, complete blood count; HPV, human papillomavirus; HSIL, high squamous intraepithelial lesions; LSIL, low squamous intraepithelial lesions; OR, odds ratio; 95% CI, 95% confidence intervals; STI, sexually transmitted infections, VLP, virus-like particle; WBC. white blood count

Introduction

Differential white blood cell (WBC) counts are routinely used as markers of overall health status as abnormal values can indicate infection, cancer, and other diseases.[1–5] More recently, WBC counts (highest versus lowest quartile) demonstrated a significant association with metabolic syndrome, hyper triglyceridemia, low HDL-cholesterol, and high fasting blood glucose.[2] Several smaller studies categorizing quantitative and qualitative T cell lymphocyte and other leukocyte measures in women with precancerous cervical lesions or cervical cancer have been performed; however, these studies were small and generated differing conclusions.[6–8] Normal ranges for CBC are quite broad with the highest value at the top of the normal range often several fold higher than the lowest value at the bottom of the normal range.[9] Because normal ranges are so broad, it is also possible that leukocyte count may serve as a crude measure of more subtle differences in the immune system and represent determinants of HPV clearance in healthy individuals.

The current study consists of a community-based population of healthy young adult women enrolled in a bivalent human papillomavirus (HPV) vaccine trial. Because participants were young adults, they were at an age when rates of sexually transmitted infections (STI) tend to peak in many populations.[10] Accordingly, a high prevalence of STIs were recorded in this population, with HPV and *chlamydia trachomatis* (CT) being the most prevalent. Thus, we investigated associations between circulating leukocyte counts and current CT infection, HPV infection and HPV-associated cervical lesions.

Materials and methods

Study Population

We used data from the Costa Rica Human papillomavirus (HPV) 16/18 vaccine trial (CVT) in Guanacaste, Costa Rica which has been previously described.[11] In brief, CVT is a community-based double-blind randomized controlled phase III trial of a virus-like particle (VLP) vaccine against HPV types 16 and 18. Women were invited to participate based on a census of the young adult female population in the region; the CVT included 7,466 healthy 18-25year old women. The

trial (NCT00128661, ClinicalTrials.gov) was reviewed and approved by the human subjects review committees of INCIENSA (Instituto Costarriciense de Investigacion y Ensenanza en Nutricion y Salud) in Costa Rica and the National Cancer Institute in the United States and all participants signed IRB-approved informed consent forms.

As part of the trial, women were interviewed to obtain information about risk factors related to HPV infection and cervical cancer and a detailed medical history and physical exam were performed by the study clinicians. After eligibility determination, and prior to vaccination, blood for complete blood count (CBC) analysis was collected and a pelvic exam was performed on sexually active participants. The processing of blood samples was performed by Trilab Sci, Liberia and CBC counts were obtained using the ABX Micros 60 hematology analyzer (ABX Diagnostics Montpellier, France). During the pelvic exam, exfoliated cells for cytology, HPV DNA and CT DNA were collected in PreservCyt solution. Processing of the PreservCyt vial for preparation of liquid-based, monolayer cytology slides, HPV and CT detection, and processing of the blood samples have been previously described.[11] Women with cytological evidence of high grade disease or cancer were referred to colposcopy where biopsies were performed for histological diagnosis of HPV-associated squamous intraepithelial lesions.

We defined HPV positivity as

i. 'Any HPV-positive' if the participant was positive for at least one HPV type.

ii. 'Carcinogenic HPV-positive' if positive for any one of 14 carcinogenic HPV types (HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68_73).

iii. 'Non-carcinogenic HPV-positive' if they were positive for any of the 11 non-carcinogenic HPV types (6/11/34/40/42/43/44/53/54/70/74) and not for a carcinogenic type by SPF10-LiPA HPV DNA detection. Cytology was categorized as normal, atypical cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesions (LSIL) or high grade squamous intraepithelial lesions (HSIL), or cancer (no cancers detected at the time of study).

Analysis

This is a cross-sectional analysis of data and information collected at the study's enrollment visit (prior to vaccination). As sexual experience is a prerequisite for STI exposure only the 5711 non-virgins, representing approximately two thirds of all enrolled women were included in the current evaluation. We analyzed the association between peripheral lymphocyte, monocyte, granulocyte, and WBC counts and with HPV-positivity at the cervix, CT positivity at the cervix, and cytologically detected ASCUS, LSIL, or HSIL. Leukocyte markers evaluated were checked for distributional properties, and categorized into tertiles of low, medium and high values. Using polytomous logistic regression models, separate analyses were performed for each marker and outcome. All analyses were two-sided. Analyses were performed using SAS and were adjusted for age, body mass index (BMI), smoking, educational grade completed, hormonal contraception, number of sex partners, number of live births, years since first sex, marital status, and CT (for all models except for CT). Women with missing values for any of the CBC counts or those with missing information for adjustment factors were excluded from the analysis. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated and P-trend analysis was used to assess statistical significance.

Results and discussion

Results

In our population of young, healthy, sexually experienced females (n=5711), 14% tested positive for CT, 42.1% tested positive for any type of HPV (non-carcinogenic and/or carcinogenic) and 35.4% tested positive for at least one type of carcinogenic HPV (Table 1). Overall, HPV-associated lesions were detected in 15.8% of women. Within this population a wide range of normal values for the immune cell parameters was observed (Table 2).

No significant associations were found between any of the peripheral leukocyte counts measured and CT. We observed some significant associations between leukocyte counts, HPV and HPV-associated lesions. In separate models, we found that higher lymphocytes counts (3rd tertile) were associated with a decreased odds of any type of HPV infection (data not shown) and separately also with decreased odds of any type of lesion (data not shown). Further analysis revealed that these associations were driven mainly by the decreased odds of carcinogenic HPV positive HSIL ($OR_{Lymphocyte\ high\ vs.\ low\ tertile}$: 0.62; 95% CI (0.43, 0.88); P-trend=0.02) in women with higher lymphocyte counts (Table 3).

Discussion

Our results suggest that higher numbers of circulating lymphocytes are associated with decreased incidence of carcinogenic HPV positive HSIL. To our knowledge this study is the first to evaluate the association between peripheral differential leukocyte counts and CT, HPV infection, and HPV-associated disease in a large population of young, healthy, well-characterized women. This suggests that a higher number of circulating lymphocyte counts may be protective from carcinogenic HPV-positive HSIL.

Past studies have demonstrated that lymphocytes appear to be directly involved in clearance of carcinogenic HPV, regression of HPV-associated lesions, and lack of lymph node metastasis in cervical cancer.[12] If an HPV infection is not adequately controlled and cleared by host lymphocytes, persistent infection and subsequent cervical disease can result. This is most evident in populations of immuno suppressed individuals, who have reduced numbers of lymphocytes resulting from HIV infection or immunosuppressive regimens required by transplantation and thus, are more susceptible to HPV-related cancers.[3,13]

Presently, aberrant leukocyte counts are used for the diagnosis of various disease states including malignancy and HIV.[3,14] Leukocyte counts are also used as prognostic markers of survival at the time of diagnosis and often, again, after administration of therapy.[5] Even though carcinogenic HPV infection and lesions are largely silent disease states, devoid of signs or symptoms of systemic infection, differences in circulating leukocyte counts and more specifically, in lymphocyte counts, may be determinants of disease progression and act as correlates of HPV immunity.

We did not find any associations between leukocyte counts and the presence of CT infection. CT and HPV both require cell-mediated immunity for pathogen clearance, though systemic leukocyte counts were not associated with the presence of CT infection. This observation could indicate that the respective bacterial and viral pathogens have different determinants of infection or indicate that our HPV-associated findings are spurious. Our study has additional limitations. This study only includes rudimentary categories of leukocytes, which lack phenotypic or functional resolution. Lastly, this study was cross-sectional in design; a future longitudinal study would allow adjustment for changes within an individual's leukocyte counts over time.

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Table 1 Demographic information for study participants

Demographic Table	Number of Participants	Percent
Total number of participants	5706	100%
Enrollment Age		
18-19	1470	25.8
20-22	2171	38.1
23-25	2065	36.2
Education Completed		
Primary school	1799	31.5
Secondary school	2922	51.2
More than secondary school	985	17.3
Smoking		
No (never)	4802	84.2
Former	537	9.4
Current	367	6.4
Marital Status		
Single	2525	44.3
Married/living as married	2999	52.6
Divorced/separated	182	3.2
Hormonal Contraception (current)		
No, <6 months	2322	18.1
Yes, 6-36 months	2349	41.2
Yes, >36 months	1035	18.1
Live Births		
0	2424	42.5
2-Jan	3014	52.8
3+	268	4.7
Number of lifetime sexual partners		
1	2420	42.4
2	1492	26.1
3	866	15.2
4+	928	16.3
Years since first sex		
0-1	691	12.1
4-Feb	2181	38.2
5+	2834	49.7
HPV*		
HPV negative (all HPV)	3283	57.9
HPV positive (non-carcinogenic types only)	381	6.7
HPV positive (carcinogenic types +/- non-carcinogenic types)	2011	35.4
Cytology*		
No pathology	4778	84.2
≤LSIL	802	14.1
≥HSIL	95	1.7
Current Chlamydia status**		
No	4869	86
Yes	793	14

*N=5675 and **N=5662 due to missing values; ≤LSIL includes ASCUS and LSIL

CT: Chlamydia Trachomatis; HPV: Human papillomavirus; HSIL: High Squamous Intraepithelial Lesions; LSIL: Low Squamous Intraepithelial Lesions

Table 2 Summary description of the various markers evaluated

Demographic Table	Number of Participants	Percent
Total number of participants	5706	100%
Enrollment Age		
18-19	1470	25.8
20-22	2171	38.1
23-25	2065	36.2
Education Completed		
Primary school	1799	31.5

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Table Continued...

Demographic Table	Number of Participants	Percent
Secondary school	2922	51.2
More than secondary school	985	17.3
Smoking		
No (never)	4802	84.2
Former	537	9.4
Current	367	6.4
Marital Status		
Single	2525	44.3
Married/living as married	2999	52.6
Divorced/separated	182	3.2
Hormonal Contraception (current)		
No, <6 months	2322	18.1
Yes, 6-36 months	2349	41.2
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HPV*		
HPV negative (all HPV)	3283	57.9
HPV positive (non-carcinogenic types only)	381	6.7
HPV positive (carcinogenic types +/- non-carcinogenic types)	2011	35.4
Cytology*		
No pathology	4778	84.2
≤LSIL	802	14.1
≥HSIL	95	1.7
Current Chlamydia status**		
No	4869	86
Yes	793	14

N=5706; *Counts reported as 103/µl

WBC: White Blood Count

Table 3 N=5695 for HPV Outcome; N=5675 for Cytology; N=5662 for Chlamydia status; All models adjusted for age, BMI, smoking, education, hormonal birth control, number of sex partners, number of live births, chlamydia (not in Chlamydia model), marital status, years since first sex; reference category is the lowest tertile of the respective markers; ≤LSIL includes ASCUS and LSIL

Granulocytes	1st tertile	2nd tertile			3rd tertile			P-trend
	N	N	crude OR	adjusted OR	N	crude OR	adjusted OR	
HPV Negative	1089	1065	1	1	1148	1	1	0.35
Non-carcinogenic HPV	126	112	0.91	0.91 (0.70, 1.20)	144	1.08	1.11 (0.86, 1.45)	
Carcinogenic HPV								
≤LSIL	562	627	1.14	1.14 (0.98, 1.33)	616	1.04	1.09 (0.94, 1.27)	
≥HSIL	77	73	0.97	0.99 (.071, 1.38)	56	0.69	0.77 (0.53, 1.10)	
Chlamydia status								
No Chlamydia	1591	1603	1	1	1675	1	1	0.44
Current Chlamydia	249	261	1.04	1.04 (0.86, 1.26)	283	1.08	1.11 (0.92, 1.35)	
Lymphocytes	1st tertile	2nd tertile			3rd tertile			P-trend
	N	N	crude OR	adjusted OR	N	crude OR	adjusted OR	
HPV Negative	842	1152	1	1	1308	1	1	0.02
Non-carcinogenic HPV	122	118	0.71	0.72 (0.55, 0.95)	141	0.74	0.80 (0.61, 1.05)	
Carcinogenic HPV								

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Table Continued...

Granulocytes	1st tertile	2nd tertile			3rd tertile		P-trend
	N	N	crude OR	adjusted OR	N	crude OR	adjusted OR
≤LSIL	514	661	0.94	0.95 (0.81, 1.11)	630	0.79	0.87 (0.75, 1.02)
≥HSIL	70	71	0.74	0.71 (0.50, 1.01)	65	0.6	0.62 (0.43, 0.88)
Chlamydia status							
No Chlamydia	1327	1698	1	1	1844	1	1 0.83
Current Chlamydia	212	291	1.07	1.05 (0.86, 1.27)	290	0.98	0.99 (0.82, 1.21)

ASCUS: Atypical Cells Of Undetermined Significance; BMI: Body Mass Index; CT: Chlamydia Trachomatis; HPV: Human papillomavirus; HSIL: High Squamous Intraepithelial Lesions; LSIL: Low Squamous Intraepithelial Lesions; OR: Odds Ratio Table 3 (continued) Multivariate association of Chlamydia trachomatis, HPV and Cytology with peripheral leukocyte markers

Table 4 N=5695 for HPV Outcome; N=5675 for Cytology; N=5662 for Chlamydia status; All models adjusted for age, BMI, smoking, education, hormonal birth control, number of sex partners, number of live births, chlamydia (not in Chlamydia model), marital status, years since first sex; reference category is the lowest tertile of the respective markers; ≤LSIL includes ASCUS and LSIL ASCUS: Atypical Cells Of Undetermined Significance; BMI: Body Mass Index; CT: Chlamydia Trachomatis; HPV: Human papillomavirus; HSIL: High Squamous Intraepithelial Lesions; LSIL: Low Squamous Intraepithelial Lesions; OR: Odds Ratio

Monocytes	1st tertile	2nd tertile			3rd tertile		P-trend
	N	N	crude OR	adjusted OR	N	crude OR	adjusted OR
Outcome							
HPV Negative	586	873	1	1	1843	1	1 0.73
Non-carcinogenic HPV	65	99	1.02	1.04 (0.75, 1.46)	218	1.07	1.11 (0.83, 1.50)
Carcinogenic HPV							
≤LSIL	341	461	0.91	0.93 (0.77, 1.12)	1003	0.93	1 (0.85, 1.18)
≥HSIL	35	57	1.09	1.12 (0.72, 1.73)	114	1.04	1.10 (0.74, 1.64)
Chlamydia status							
No Chlamydia	890	1292	1	1	2687	1	1 0.09
Current Chlamydia	134	190	0.98	0.98 (0.77, 1.24)	469	1.16	1.18 (0.95, 1.46)
WBC	1st tertile	2nd tertile			3rd tertile		P-trend
	N	N	crude OR	adjusted OR	N	crude OR	adjusted OR
Outcome							
HPV Negative	1030	1106	1	1	1166	1	1 0.51
Non-carcinogenic HPV	123	123	0.93	0.94 (0.72, 1.23)	136	0.98	1.03 (0.79, 1.35)
Carcinogenic HPV							
≤LSIL	578	581	0.94	0.95 (0.82, 1.11)	646	0.99	1.08 (0.93, 1.26)
≥HSIL	80	71	0.83	0.82 (0.59, 1.15)	55	0.61	0.68 (0.47, 0.98)
Chlamydia status							
No Chlamydia	1547	1623	1	1	1699	1	1 0.1
Current Chlamydia	248	248	0.95	0.93 (0.77, 1.13)	297	1.09	1.13 (0.94, 1.37)

Conclusion

Differential CBC counts as an indicator of disease risk warrants replication and further exploration of lymphocyte subsets in HPV infection and cervical disease. Comparison of the localized and systemic immune response to infection is likely to be fruitful.[15] as analyses of the infiltrate of leukocytes and other immune cells at the cervix during the progression of persistent carcinogenic HPV infection may better identify characteristics of protective and permissive immune responses in HPV-associated cervical disease.

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Conflicts of interest

Author declares there are no conflicts of interest.

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