Increased Incidence of Cervical Intraepithelial Neoplasia in Women with Systemic Lupus Erythematosus Treated with Intravenous Cyclophosphamide

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ABSTRACT. ***Objective.*** To determine if the incidence of cervical intraepithelial neoplasia (CIN) is increased in immunosuppressed women with systemic lupus erythematosus (SLE).

Methods. Women with SLE were consecutively recruited from University of Michigan outpatient rheumatology clinics. Women with abnormal cervical smears at screening were excluded. Cervical smears were obtained at baseline and at 3 and 7 years. Cervical biopsies confirmed cytologic abnormalities (CIN I–III), and were scored by pathologists in blinded fashion. Data were analyzed according to treatment group: (1) prednisone; (2) azathioprine (AZA); (3) intravenous cyclophosphamide (IVCYC); and (4) IVCYC + AZA + prednisone.

Results. Sixty-one of 89 women screened were eligible for enrollment. The overall 3-year incidence of CIN was 9.8%. Stratified by treatment group, the 3-year incidence of CIN was 0/23 (0%) in prednisone treated patients, 0/4 (0%) in AZA treated patients, 2/8 (25%) in IVCYC treated patients, and 4/26 (15%) in CYC + AZA + prednisone treated patients. A dose relationship was observed between cumulative IVCYC exposure and CIN; each increase of 1 g of IVCYC exposure corresponded to a 13% increased risk of CIN ($p = 0.04$). At 7 years, 45 patients remained under followup and 6 patients had died of unrelated causes. No cases of CIN were observed at 7 years, although there were 2 cases of atypical squamous cells of unknown significance and one case of condyloma.

Conclusion. IVCYC + prednisone therapy for SLE is significantly associated with development of CIN. (J Rheumatol 2004;31:1763–7)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS CERVICAL INTRAEPITHELIAL NEOPLASIA
CERVICAL SMEAR IMMUNOSUPPRESSION CYCLOPHOSPHAMIDE AZATHIOPRINE

The occurrence of malignancies in patients with systemic lupus erythematosus (SLE) has been extensively studied[1-9]. Whether patients with SLE are at greater risk of developing cancer during the course of their disease and the contribution of immunosuppressive agents to this risk remains controversial. Several studies have suggested an increased incidence of neoplasms in patients with SLE[1-5,10,11]. A longitudinal study of 205 patients from the Finnish Cancer Registry[5], a cohort study of 616 women from the Chicago Lupus Cohort linked with the Illinois State Cancer Registry[4], and a study of 209 women from Allegheny County, Pennsylvania[7], suggest relative risks of developing cancer in SLE of 2.6, 2.0, and 1.4, respectively. Others have found no overall increase in incidence of malignancies in SLE compared to the general population[6-9].

Immunosuppressive therapy and impaired immunosurveillance have been suggested as predisposing factors in the occurrence of a variety of malignancies in lupus patients[1–3,5,11]. Similarly, studies in patients with rheumatoid arthritis treated with cyclophosphamide (CYC) or azathioprine (AZA)[12-15], as well as renal transplant patients treated with AZA[16,17], have shown an association with secondary neoplasms.

Cervical intraepithelial neoplasia (CIN), a term encompassing premalignant and malignant lesions[18], has been reported to be increased in lupus patients[1-3,8,10,19,20] and associated with immunosuppression. Gourley and Bateman

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observed increased CIN in association with intravenous (IV) CYC[19,20]. Nyberg, *et al* found that 17 of 19 (89%) lupus patients with CIN had received AZA therapy[10]. These results are consistent with observations in the renal transplant population showing increased rates of CIN in association with AZA use[16,17].

Monthly IVCYC therapy, which is widely used for many severe forms of lupus, has multiple putatively immunosuppressive properties in SLE patients that might predispose to an increased incidence of CIN[21]. Despite widespread use of IVCYC, longterm studies regarding the associated risks of CIN in lupus patients are lacking. To address this issue, we prospectively evaluated the incidence of CIN over a 3-year period in women treated with IVCYC and/or AZA plus prednisone compared to controls treated with prednisone alone, with followup after 7 years.

MATERIALS AND METHODS

Subjects and design. Women with SLE followed at the University of Michigan Outpatient Rheumatology Clinics were consecutively offered enrollment in this prospective study. This study was approved by the University of Michigan Institutional Review Board and written informed consent was required for study participation. Inclusion criteria for the study were age ≥ 18 years and a diagnosis of SLE according to American College of Rheumatology (ACR) criteria[22,23]. Patients treated with oral CYC were excluded from this study for the following reasons: (1) there were not enough patients in this group to analyze separately; (2) the higher cumulative dosing of oral CYC in this group may have biased results; and (3) the immunosuppressive actions of daily oral CYC may differ from those of monthly IVCYC[21]. Patients with abnormal cervical smears at screening were also excluded, as were patients with no previous sexual exposure.

Baseline cervical smears were performed at study entry, and subsequent cervical smears were performed annually, or more frequently as practice dictated. Longterm followup of cervical smear data was also evaluated after 7 years. Colposcopy and cervical biopsy were performed to confirm any cytologic abnormality and the results were classified as abnormal only after biopsy confirmation. Pathologists reviewed all cervical smears and biopsies in blinded fashion. Patients with CIN were treated and followed by their gynecologists as appropriate.

Pathologic studies. Biopsies were classified according to standard criteria for classification of cervical histology based on the classification first described by Richart[24] and Reagan[25], and the cervical smears were categorized by the 1988 Bethesda System for Reporting Cervical/Vaginal Cytological Diagnoses[26,27]. Lesions histologically categorized as CIN I consist of cellular abnormalities confined to the basal third of the cervical epithelium, which correspond to low grade squamous intraepithelial lesions in the Bethesda System. CIN II lesions have cellular abnormalities confined to the basal 2/3 of the epithelium, and CIN III lesions are those with cellular abnormalities encompassing > 2/3 of the epithelial thickness, which includes full-thickness lesions (carcinoma *in situ*). CIN II and CIN III are considered high grade squamous intraepithelial lesions in the Bethesda System.

Statistical analysis. Data management and analysis were performed using Stata™ version 7.0 (Stata Corporation, College Station, TX, USA). Patients were stratified according to the treatment administered for lupus. For all comparisons, the patients who were treated with prednisone but had no current or prior exposure to CYC, AZA, or methotrexate (MTX) served as the control group. Descriptive statistics were obtained to characterize the population at baseline. Continuous variables were summarized using means and standard deviations (SD), and significance was tested by 2-sample t test. Categorical variables were summarized by frequency counts and percentages, and Fisher's exact test was used to test for significance. The risk of developing incident CIN was calculated for each treatment group, and significance was tested by chi-square analysis. Logistic regression was used to examine the association between CYC dose and development of CIN. The standardized incidence ratio was computed by dividing the observed number of cases of CIN in this population by the number expected based on national data[28]. Confidence intervals (95% CI) were calculated based on the Poisson distribution[29].

RESULTS

Eighty-nine women were identified for the study. Of these, 6 were treated with oral CYC and 4 were treated with MTX and were therefore excluded. Ten patients, including 9 with prior or current immunosuppression with AZA and/or CYC, were excluded due to abnormal baseline cervical smears (CIN I–III). Eight additional patients were excluded as follows: 3 did not obtain baseline cervical smears, one had no prior history of sexual activity, one did not meet ACR criteria for SLE, one had undergone prior hysterectomy, and 2 received no treatment with either prednisone or cytotoxic agents. The remaining 61 women with SLE were included, all of whom had normal baseline cervical cytology. Baseline characteristics of the study population are described in Table 1.

Cervical cytology data at the 3-year followup are shown in Table 2. In this population, the incidence of CIN was 0% among patients receiving prednisone only or AZA + prednisone. Patients who had received either CYC alone (+ prednisone) or CYC in combination with AZA and prednisone had a significantly higher risk of developing CIN compared to the control group of patients receiving prednisone alone (incidence of 0.25, p = 0.0132, and 0.15, p = 0.0497, respectively). The only high grade lesion was found in the combination group. Results are expressed in terms of absolute risk (incidence) rather than risk ratios since there was no incident case of CIN in the control group.

A subset analysis of the 34 patients who received CYC revealed a positive relationship between cumulative CYC

Table 1. Baseline characteristics of study population (n = 61).

	Prednisone, n = 23	Cytotoxic Treatment, n = 38	p*
Age, mean (SD) yrs	33.5 (9.1)	32.9 (8.0)	0.788
Disease duration, mean (SD) yrs	9.5 (5.9)	9.9 (7.1)	0.812
Race, n (%)			
Caucasian	22 (95.7)	30 (79.0)	0.313
African American	1 (4.4)	4 (10.5)	
Hispanic	0	2 (5.3)	
Asian	0	2 (5.3)	
Treatment, n (%)			
Prednisone only	23 (100)	—	—
AZA + Pred	—	4 (10.5)	
CYC + Pred	—	8 (21.1)	
CYC + AZA + Pred	—	26 (68.4)	

Pred: prednisone; AZA: azathioprine; CYC: cyclophosphamide.
* Cytotoxic treatment vs prednisone.

Table 2. Cervical cytology at 3-year followup, according to treatment group.

Treatment	Mean CYC Dose ± SD, g	Normal	Low Grade (CIN I)	High Grade (CIN II-III)	Incidence of CIN I-III*, %	p**
P	—	23	0	0	0	—
AZA + Pred	—	4	0	0	0	—
CYC + Pred	17.4 ± 15.8	6	2	0	25	0.0132
CYC + AZA + Pred	13.9 ± 8.9	22	3	1	15	0.0497

Pred: prednisone; AZA: azathioprine; CYC: cyclophosphamide. * Incidence (risk) is reported rather than risk ratios because the risk in the referent category equals zero. ** Referent category is prednisone only.

dose and development of CIN. When controlling for age, each 1 g increase in cumulative CYC exposure corresponded to a 13% increase in risk of CIN (risk ratio 1.13, p = 0.04). The mean (± SD) cumulative CYC exposure in this group was 14.6 ± 10.5 g.

Longterm followup was performed at 7 years. There were 6 deaths (2 lymphoma, one sepsis, one interstitial lung disease, one cardiovascular disease, and one related to endstage renal disease). Data were available for 45 of the remaining 61 original patients (74%), and revealed abnormal cervical smears in 3 of these 45 patients (7%). Two of these 3 patients had normal cervical smears at 3 years; at followup one patient had atypical squamous cells of unknown significance (ASCUS) and the other had condyloma. The third patient had CIN I at 3 years and ASCUS at 7 years. One patient had CIN III at 3 years and resolution of the cervical abnormality at 7 years; however, she developed vulvar disease and underwent vulvectomy.

DISCUSSION

In our study of women with lupus, we found the incidence of CIN to be 9.8% over a 3-year period. By comparison, in one of the largest and most generalizable studies, the National Breast and Cervical Cancer Early Detection Program (1991–95), CIN was found in 15,119 of 472,188 women (3.2%)[28]. Based on these data, the standardized incidence ratio for CIN in our study was 3.08 (95% CI 1.13, 6.70), which represents significantly elevated incidence of CIN in this cohort compared to the general population in the US. The majority of cervical abnormalities in this population were observed within the initial 3-year period, and most did not persist at 7-year followup. Studies in the general population have similarly reported up to 74% spontaneous regression of cervical abnormalities to normal cervical smears[18,30].

While the increased rate of CIN in the lupus population has been previously documented, our study focused on the role of immunosuppression, and the relationship of CIN to cumulative dosing of immunosuppressive therapy. We found a strong association between the development of cervical abnormalities and the use of IVCYC plus prednisone, with or without AZA, in this population. The data do not show that AZA is necessarily more benign than IVCYC in relationship to the risk of CIN since only 4 patients received AZA alone, resulting in limited statistical power to detect such an association.

Evidence in favor of IVCYC as a potential causative agent in the development of cervical disease is supported by several findings. First, we found a significant dose relationship between the cumulative IVCYC exposure and the development of CIN. Over the initial 3-year period, a 13% increase in risk of progression from normal to CIN I–III was observed for each gram of IVCYC administered. Second, our choice of a control group receiving some degree of immunosuppression (versus lack of any treatment) shows that the increased risk of cervical abnormalities is not consistent across classes of immunosuppressive drugs. Indeed, no cervical abnormalities occurred in the group treated with prednisone alone. Third, because this prospective study looked at only incident cases of CIN, we have established a temporal relationship between the use of IVCYC and development of cervical abnormalities.

The potential role of IVCYC in the pathogenesis of CIN could be explained through direct mutagenic actions of the drug and its metabolites, or indirectly, through its effects as a potent immunosuppressive. Identifying the precise mechanisms for CYC is complicated by the presence of multiple active metabolites, many of which may have differing immunosuppressive and/or mutagenic effects[31]. The carcinogenic potential of CYC has been implicated in a number of malignancies, such as neoplasia of the urinary tract, skin, and bone marrow[21]. Notably, risk of malignancy correlates with cumulative CYC exposure[31]. Additionally, the degree of immunosuppression, which may also be a function of increasing dose, is another plausible mechanism by which CYC could promote progression to CIN. With immunosuppression in general, there is an increased susceptibility to infection or reactivation of human papillomavirus (HPV) infection[32]. HPV is a known risk factor for cervical abnormalities, and certain strains have proven more likely to cause CIN than others[33]. It is interesting that in transplant patients, another group receiving immunosuppressive therapy, preferential expression of pathogenic strains of HPV has been exhibited[34]. Similarly, in patients with human

immunodeficiency virus, the rate of HPV infection is related to the level of immunosuppression, as measured by decreasing CD4 counts[35-37].

While the above data support the role of CYC as a potential contributory agent in CIN, a number of other host and environmental factors have been implicated in its etiology, such as sexual behavior and smoking[38,39]. To confirm the role of CYC as an independent risk factor for CIN, future studies in patients with SLE should incorporate HPV serotyping and other risk factors. Further, since treatment with IVCYC is inherently indicative of more severe disease activity, it is possible that the results we observed were in part related to increased SLE activity. Therefore, future studies would also benefit from standardized assessment of lupus activity.

Until such studies are performed, our observations of high incidence of CIN, as well as the strong association between IVCYC and development of CIN in this population, highlight the importance of careful screening of women with lupus, particularly those receiving immunosuppressive therapy. By inference, the risk for other HPV-related gynecologic lesions may also be increased in this setting. The dose-response relationship we observed further implicates IVCYC in the pathogenesis of CIN, and underscores the necessity of limiting the cumulative dose of CYC to reduce end-organ damage. Unfortunately, it is not certain that the substitution of alternative immunosuppressive agents, such as AZA, will reduce the risk of developing CIN. Until safer treatments for severe lupus become available, heightened surveillance is indicated to reduce the longterm risk of developing cervical cancer.

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