Human Papillomavirus Infection and Cytological Atypia in Female Allogeneic Hematopoietic Stem Cell Transplantation Recipients

Adela Saco, MD,[1,2] Sara Carbonell, MD,[2,3] Natalia Rakislova, MD,[1,4] Isabel Matas, MD,[5] Silvia Alòs, BSc,[1] Sandra Hoya, BSc,[1] María Suárez-Lledó, MD,[2,3] Katarzyna Darecka, MD,[1] Lia Sisuashvili, MD,[1] Lorena Marimon, BSc,[1,4] Naiara Vega, BSc,[1] Roser Esteve, BSc,[1] Carmen Martínez, MD,[2,3] Cristina Martí, MD,[5] Ariel Glickman, MD,[5] Olga Balagué, MD,[1,2] Aureli Torne, MD,[2,5] Jaume Ordi, MD,[1,4] and Marta del Pino, MD[2,5]

Background. Female recipients of allogeneic hematopoietic stem cell transplantation are at high risk of developing human papillomavirus (HPV)-associated lesions and (pre)cancer. We describe the results of a cervical cancer screening program in these women. **Methods.** From 2010 to 2022, 70 female recipients of allogeneic hematopoietic stem cell transplantation in our institution entered a standardized protocol of gynecological evaluation. HPV testing, Papanicolaou smear, and thorough gynecological examinations were conducted in all the women. **Results.** The cumulative prevalence of HPV infection was 21.4% (15/70). Ten of 70 women (14.3%) had a positive HPV test result in the first gynecological evaluation and 5 additional women (7.1%) became positive during follow-up. Thirteen women (18.5%) presented cytohistological lesions (3 high-grade lesions and 10 low-grade lesions). Twenty-nine women (41.4%) showed HPV-negative reactive atypical abnormalities related to the conditioning treatment, which closely mimicked HPV-associated lesions, which spontaneously disappeared during follow-up. **Conclusions.** Gynecological evaluation should be maintained over time, as a significant proportion of these women may become HPV positive during follow-up. Reactive benign, atypical changes related to the treatment, which closely mimic HPV-associated lesions, are a frequent finding in these women. HPV testing is a key tool for the evaluation of these patients, as it allows for identifying women at risk and excluding cytological mimickers.

(*Transplantation* 2025;00: 00–00).

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only potentially curative treatment for several malignant and nonmalignant hematologic disorders. However, allo-HSCT is associated with several complications. These complications may be linked to the consequences of the conditioning treatment, the immunologic reaction against the recipient tissues raised by the donor cells (graft-versus-host disease [GvHD]), or the immunosuppression caused by this treatment. A well-known consequence of this latter effect is an increase in the risk of infections due to carcinogenic microorganisms, which result in an increased incidence of secondary tumors.[1] Indeed, patients who have undergone allo-HSCT are at high risk of developing human papillomavirus (HPV)-associated (pre)cancer,[2-9] with a reported incidence

Received 5 July 2024. Revision received 15 October 2024.

Accepted 5 November 2024.

[1] Department of Pathology, Hospital Clínic, University of Barcelona, Barcelona, Spain.

[2] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

[3] Long-Term Follow-up Unit, Hematopoietic Stem Cell Transplantation Unit, Hematology Department, Institute of Cancer and Blood Disease (ICAMS), Hospital Clinic, Barcelona, Spain.

[4] Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain.

[5] Department of Gynecology, Institute Clinic of Gynecology, Obstetrics, and Neonatology, Hospital Clínic, University of Barcelona, Barcelona, Spain.

This study was funded by Instituto de Salud Carlos III (ISCIII) through the project PI21/00928 and co-funded by the European Union.

The authors declare no conflicts of interest.

A.S., J.O., and M.d.P. participated in the concept and design of the study. M.d.P., I.M., C.M., A.G., M.S.-L., S.C., and C.M. participated in the recruitment of patients and data collection and supervised the clinical aspects. A.S., N.R., K.D., L.S., and J.O. participated in the cytological and histological diagnosis. S.A., S.H., N.V., and R.E. provided technical support and participated in the cytological screening of the Papanicolaou tests. L.M. participated in human papillomavirus testing. A.S. and M.d.P. performed the formal analysis. A.S. drafted the first version of the article, with significant contributions from M.d.P., A.T., O.B., and J.O., and incorporated all the modifications recommended by the other authors. All authors contributed to critical review of the article, approved the final version, and read and agreed to the published version of the article.

Correspondence: Marta del Pino, MD, Department of Gynecology, Institute Clinic of Gynecology, Obstetrics, and Neonatology, Hospital Clínic. University of Barcelona, Villarroel 170, 08036, Barcelona, Spain. (mdelpino@clinic.cat).

ISSN: 0041-1337/20/0000-00

of cervical cancer (CC) 13 times higher than the incidence in the general population.[1,10] For this reason, scientific societies recommend gynecological evaluations with CC screening using HPV testing and/or Papanicolaou (Pap) smear in all female allo-HSCT recipients.[1,11-13] Despite these recommendations, several studies have shown suboptimal screening rates in allo-HSCT recipients,[14,15] and very few studies have analyzed CC screening results in these women.

In recent years, few studies have reported that, in addition to abnormal cytological results due to HPV infection, allo-HSCT female recipients may show cytological abnormalities that are not related to the virus but closely mimic HPV-associated lesions, representing a major challenge for pathologists. Indeed, some alkylating agents used in the conditioning regimen for allo-HSCT, particularly busulfan, have been associated with epithelial abnormalities in different anatomic areas, including the uterine cervix.[6,16-18] In a previous study, we reported a series of patients showing squamous cells with atypical changes associated with busulfan in the cervical smear. These changes resembled low-grade squamous intraepithelial lesions (LSILs) or even high-grade squamous intraepithelial lesions (HSILs).[16] These cytological abnormalities may be a source of confusion in the screening of CC in these women and may result in difficulties in clinical management as well as an increase in the risk of overdiagnosis and overtreatment.

In the present series, we describe the results of a CC screening program in this subset of high-risk women who attended a Gynecology Department from 2010 to 2023.

MATERIALS AND METHODS

Study Design and Inclusion Criteria

This was a prospective study conducted at the Gynecologic Oncology Unit of the Hospital Clinic of Barcelona, a referral center for hematologic diseases.

From 2010 to 2016, gynecological evaluation in the Department of Gynecology of the Hospital Clínic was offered to allo-HSCT-treated women presenting symptoms suggesting GvHD in the genital area. Since 2017, all allo-HSCT female recipients have been offered gynecological control in the Gynecology Oncology Unit as a part of a multifaceted posttransplantation control program study. All the women undergoing gynecological control in our center between 2010 and 2023 were included.

Study Protocol

The standard protocol for gynecological evaluation in allo-HSCT patients included a thorough gynecological history, blood analysis with hormone levels, evaluation of bone density, assessment of signs and symptoms of GvHD in the genital tract, and CC screening based on HPV testing, and cervical liquid-based cytology (Pap smear), with colposcopy and biopsy if indicated.

The medical records of all the women included in the study were examined to search for previous gynecological examinations.

CC Screening: HPV Testing and Liquid-based Cytology

Cervical samples were collected from all the women using a cytological brush and stored in PreservCyt solution (Hologic, Marlborough, MA). HPV testing was performed in all the samples using 2 tests: Cobas HPV test (Cobas 4800; Roche Molecular Diagnostics, Pleasanton, CA) and Allplex HPV28 Assay (Seegene, Seoul, South Korea). Cobas test detects 14 high-risk HPV types and provides specific genotyping for HPV16 and HPV18, whereas high-risk HPV types other than 16/18, which include HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 are reported as a pool. Allplex HPV28 Assay detects and differentiates 9 low-risk HPV types: HPV6, 11, 40, 42, 43, 44, 54, 61, 70, and 19 high-risk HPV genotypes: HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82. The Cobas test was used for diagnostic purposes, whereas the Allplex Assay was used to detect low-risk HPV types and determine specific high-risk genotypes other than HPV16/18.

Thin-layer slides for Pap smear evaluation were prepared with a ThinPrep T2000 slide processor (Hologic). Pap smears were stained using the Pap method and reported using Bethesda nomenclature.[19]

Treatment-related atypia was diagnosed on the basis of the presence of atypical squamous cells in women with an HPV-negative test Pap smear result. These findings included nuclear enlargement, hyperchromatism, and a slightly irregular shape. The amount of cytoplasm was variable, ranging from cells with large cytoplasm simulating LSILs to cells with scant cytoplasm and even naked atypical nuclei simulating HSILs. These cells were usually mixed with cells showing cytomegaly and enlarged nuclei with smudged chromatin, suggesting reparative changes or cytological abnormalities associated with chemoradiation therapy[16] (Figure 1).

Colposcopic Evaluation and Histological Diagnosis

All women with a positive HPV test or abnormal Pap smear result were referred for colposcopic evaluation. The colposcopic examination was performed using an Olympus EvisExera II CV-180 (Tokyo, Japan).[20] Briefly, 5% acetic acid was applied to the cervix for 1–2 min using cotton balls. To detect "fast fader" lesions, the acetic acid is repeatedly reapplied. Finally, an iodine solution was applied, allowing cervical and vagina evaluation. Colposcopy impressions were described according to the classification of the International Federation of Cervical Pathology and Colposcopy.[21,22] During the colposcopy procedure, 1–4 colposcopy-directed biopsies of any abnormal area of the cervix were obtained.[23] Additionally, endocervical curettage was performed in all women with an abnormal screening test and noncompletely visible transformation zone.

All biopsies and endocervical curettages were fixed in 10% neutral buffered formalin and embedded in paraffin following routine procedures. Sections measuring 4 μm in thickness were stained with hematoxylin and eosin. p16 immunohistochemical staining (CINtec Histology Kit; mtm-Roche Laboratories, Heidelberg, Germany) was performed in the histological samples obtained.[24] Biopsy specimens were classified as normal, LSILs, or HSILs according

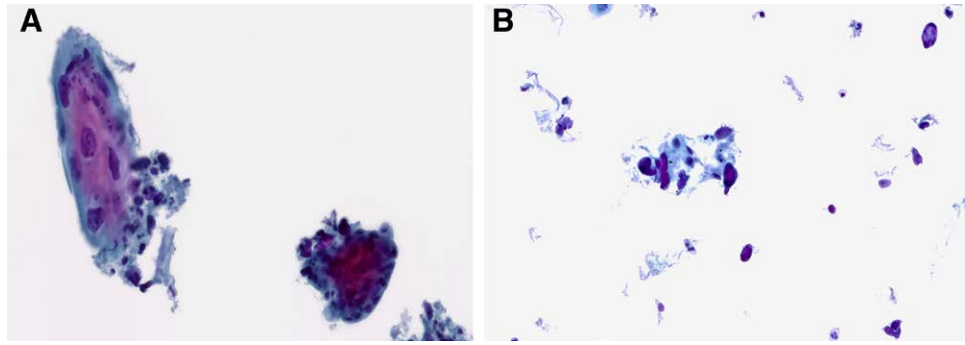

FIGURE 1. Reactive atypia related to treatment in the Papanicolaou test. A, Atypical squamous cells with nuclear enlargement and irregular shape with a large cytoplasm mimicking LSIL (×400). B, Atypical squamous cells with nuclear enlargement and hyperchromatism showing scan cytoplasm and naked nuclei mimicking an HSIL (×400). HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

to the 2020 World Health Organization classification.[25] The histological diagnosis was based on hematoxylin and eosin criteria; however, p16 block staining was required to diagnose HSILs.[26]

GvHD Assessment

The GvHD prophylaxis regimen consisted of a combination of calcineurin inhibitors, specifically cyclosporine or tacrolimus, in conjunction with methotrexate (if myeloablative conditioning regimens were used) or mofetil mycophenolate (if reduced conditioning regimens were used). Immunosuppressive drugs were maintained at an effective dosage until day +180 and then progressively tapered up to day +250 in the absence of GvHD. From 2016 until the present, high-dose posttransplant cyclophosphamide, administered on days +3 and +4, and tacrolimus, initiated on day +5 after transplantation, have been used. In this context, tacrolimus is maintained at a therapeutic level until day +90 and then progressively tapered down progressively up to day +180 in the absence of GvHD. No patient received antithymocyte globulin.

The diagnosis and grading of acute and chronic GvHD were conducted in accordance with the Mount Sinai Acute GvHD International Consortium criteria[27] and the 2014 National Institute of Health Consensus Criteria,[28,29] respectively. Patients with GvHD were managed in a uniform manner throughout the study period, with corticosteroids serving as the primary therapeutic option for GvHD.

On each visit, women underwent a comprehensive evaluation of the lower genital tract to identify GvHD signs or symptoms. According to the National Institute of Health Consensus Criteria, lichen planus-like features and vaginal scarring or stenosis are diagnostic signs of genital chronic GvHD that do not require biopsy. Erosions, fissures, and ulcers are distinctive signs requiring the exclusion of infection, drug effects, malignancy, or other causes by clinical and histological evaluation. Pain or burning, dysuria, redness and swelling, perineal and perianal soreness, dryness, and dyspareunia, despite adequate systemic and/or topical hormone therapy, are considered nonspecific symptoms of female genital chronic GvHD.[28,30]

Follow-up Strategy

Follow-up visits were scheduled according to the first gynecological evaluation test results.[20,31] Women with a negative HPV testing result or with HPV infection but no histological HSIL were scheduled for regular visits, which included HPV testing and Pap smear. If either of these tests were positive, a colposcopy evaluation was performed. Women with a histological HSIL were referred to treatment. In women diagnosed with treatment-related atypia, yearly visits with HPV testing and Pap smear were also scheduled.

Statistical Methods

The SPSS version 28.0 statistical package (SPSS, Chicago, IL) was used to perform the data analyses. Categorical variables are shown as absolute numbers and percentages and compared with the chi-square or Fisher exact test. Quantitative variables are shown as means ± SDs.

The study was approved by the ethics committee of the hospital clinic (HCB/2021/0288).

RESULTS

One hundred thirteen women underwent allo-HSCT from January 2010 to May 2023, 28 (24.8%) from 2010 to 2016, and 85 (75.2%) from 2017 to 2023. Seventy women (16 during the first period and 54 during the second period) accepted gynecological care at our institution and were included in the study. No information on the gynecological status of the 43 women who refused the gynecological controls was available. The mean age of the patients was 48 ± 14 y. The time between allo-HSCT and the first gynecological evaluation ranged from 2 to 72 mo (mean 14 ± 13.5 mo).

The hematologic diseases that led to allo-HSCT were acute myeloid leukemia in 38 women (54.3%), acute lymphoblastic leukemia in 11 (15.7%), myelodysplastic syndrome in 9 (12.9%), non-Hodgkin lymphoma in 7 (10.0%), Hodgkin lymphoma in 3 (4.3%), and plasma cell leukemia and aplastic anemia in 1 woman each (1.4% each).

The conditioning treatment was busulfan and fludarabine in 35 patients (50.0%); busulfan and cyclophosphamide in 10 (14.3%); fludarabine and total body irradiation in 10 (14.3%); thiotepa, busulfan, and fludarabine in 4 (5.7%); fludarabine and cyclophosphamide in 3 (4.3%); fludarabine and melphalan in 3 (4.3%); cyclophosphamide and total body irradiation in 2 (2.9%); busulfan and melphalan in 1; idarubicin in

1; and idarubicin, cytarabine, fludarabine, and busulfan in 1 (1.4% each). Overall, the conditioning treatment for allo-HSCT included fludarabine in 56 of 70 (80%), busulfan in 51 of 70 (72.9%), cyclophosphamide in 15 of 70 (21.4%), melphalan in 5 of 70 (7.1%), thiotepa in 4 of 70 (5.7%), idarubicin in 2 of 70 (2.9%), and cytarabine in 1 of 70 (1.4%) treatments.

HPV Testing and Pap Smear Results at the First Gynecological Evaluation

Table 1 shows the HPV testing and genotyping results obtained with the 2 techniques used in the study, as well as the cytological results of the first gynecological evaluation after allo-HSCT in the 70 patients included in the study.

Ten of 70 patients (14.3%) had a positive HPV result with the routine test (Cobas) in the first gynecological evaluation: 1 had HPV16, 1 had HPV18, and 8 showed high-risk HPV type/s other than 16/18. Among these 10 HPV-positive women, 7 (70%) showed abnormal cytological results: 5 had LSILs and 2 had HSILs. The Allplex Assay showed that 2 patients (1 with LSILs and 1 with HSILs) had coinfection by a low-risk HPV type.

Sixty women (60/70; 85.7%) showed a negative HPV result with the routine test in the first gynecological evaluation. Among these, 29 (29/60; 48.3%; 29/70 of the overall series, 41.4%) showed atypical reactive changes in the Pap smear that were considered to be related to the treatment. Thus, a total of 36 women showed atypical changes in the Pap smear, of which 7 (19.4%) were associated with HPV, whereas 29 (80.6%) were related to the treatment. The mean time from allo-HSCT to the abnormal Pap smear was 10 ± 7 mo (range, 2–31 mo). None of these patients with reactive atypia had received radiation therapy as part of the conditioning treatment. A low-risk HPV type (HPV42) was detected in 1 of 29 patients (3.4%) with treatment-related atypia. Another patient in this group was positive for a high-risk HPV type not included in the Cobas test (HPV82). Thus, after testing all cases with treatment-related atypia, HPV infection by high-risk or low-risk HPV types could be excluded in 27 of 29 patients (93.1%). Low-risk HPV types were also detected in a similar percentage of the women with a negative Pap smear and a negative Cobas test result (1/31; 3.2%). In this latter group of women, another patient was positive for a high-risk HPV type not detected by the Cobas test (HPV73).

Table 2 shows the clinical and pathological features of women with a positive HPV test at the first evaluation. All 5 women with cytological LSILs had a normal colposcopy and a negative biopsy. The 2 cytological HSILs were histologically confirmed: one presented a cervical and the second a vaginal lesion.

All women with treatment-related reactive atypia except 1 (28/29; 96.6%) had received busulfan alone or in combination with other drugs as the conditioning treatment. Reactive atypia associated with treatment was identified in 28 of 51 women (54.9%) who received busulfan; in 24 of 56 (42.9%) who received fludarabine; in 5 of 15 (33.3%) who received cyclophosphamide; in 1 of 5 (20%) who received melphalan; and in no patients who had received thiotepa, idarubicin, or cytarabine. The differences in terms of the percentage of women receiving any of these drugs who developed atypia were not significant. The mean time from allo-HSCT to the first gynecological evaluation was 9.6 ± 7.4 mo (range, 2–31 mo). All the women showed normal colposcopy in the first gynecological evaluation (29/29).

GvHD and HPV Infection

Thirty-five of 70 women (50%) were diagnosed with GvHD and in 13 of these 35 women (37.1%), the genital tract was involved.

There were no differences in terms of the prevalence of HPV infection between women with and without GvHD (5/35; 14.3% versus 5/35; 14.3%, respectively; $P = 1$). Neither were there any differences between these 2 groups in terms of cytological SIL (2/35; 5.7% versus 5/35; 14.3%, $P = 0.428$) or in women presenting reactive atypia related to treatment (14/29; 48.3% versus 21/41, 51.2%; $P = 1$).

CC Screening History Before Allo-HSCT

Among the 70 women included in the study, 6 (8.6%) were younger than 25 y at the time of HSCT and, therefore, had not started CC screening. Thirty women (42.8%)

TABLE 1.

HPV test and Pap smear results obtained in the first evaluation after allo-HSCT in the 70 patients included in the study

	HPV testing results																	
	Cobas HPV test								Allplex HPV28 assay									
Pap smear result	Negative (n = 60)		hrHPV16 (n = 1)		hrHPV18 (n = 1)		hrHPV non16/18 (n = 8)		Negative (n = 60)		hrHPV16 (n = 1)		hrHPV18 (n = 1)		hrHPV non16/18[a] (n = 11)		lrHPV (n = 4)		Total	
Negative	31	(91.2)	0	(0)	0	(0)	3	(8.8)	29	(85.3)	0	(0)	0	(0)	4	(11.8)	1	(2.9)	34	(100)
LSIL	0	(0)	1	(20.0)	0	(0)	4	(80.0)	0	(0)	1[b]	(20.0)	0	(0)	5[b]	(100)	1[b]	(20.0)	5	(100)
HSIL	0	(0)	0	(0)	1	(50.0)	1	(50.0)	0	(0)	0	(0)	1	(50.0)	1[c]	(50.0)	1[c]	(50.0)	2	(100)
Reactive atypia	29	(100)	0	(0)	0	(0)	0	(0)	27	(93.2)	0	(0)	0	(0)	1	(3.4)	1	(3.4)	29	(100)

Data are presented as absolute numbers and percentages.

[a]Three patients had a new HPV type not detected by the Cobas test (2 patients had HPV82 and 1 patient had HPV73).

[b]One patient had a multiple infection comprising high-risk HPV16 and HPV82 and low-risk HPV42 and HPV54.

[c]One patient had a multiple infection comprising high-risk HPV31, 53, and 73 and low-risk HPV42, 44, and 54.

allo-HSCT, allogeneic hematopoietic stem cell transplantation; HPV, human papillomavirus; hrHPV, high-risk HPV; HSIL, high-grade squamous intraepithelial lesion; lrHPV, low-risk HPV; LSIL, low-grade squamous intraepithelial lesion; Pap, Papanicolaou; Reactive atypia, reactive atypia associated with treatment.

TABLE 2.

Clinical and pathological features of the patients who underwent allo-HSCT and had a positive HPV test result in the first checkup after allo-HSCT

								HPV testing (first evaluation)							
Case	Age, y	Previous screening	Conditioning treatment	GvHD genital/ elsewhere	Active GvHD	Immunosuppressor drugs	Months after allo-HSCT (first evaluation)	Cobas	Allplex	Pap smear (first evaluation)	Colposcopy (first evaluation)	Biopsy (first evaluation)	Months after allo-HSCT (last follow-up)	HPV testing (last follow-up)	Pap smear (last follow-up)
1	53	No	Flu/Mel	Yes/yes	Chronic	Treatment with mofetil mycophenolate	70	Non 16/18	56	LSIL	Normal	Negative	112	Non 16/18 (56)	LSIL
2	57	HSIL	Bu/Flu	No/yes	No	Prophylaxis with Cyclosporine A	4	Non 16/18	56	LSIL	Normal	Negative	Lost to follow-up		
3	52	Normal	Bu/Flu	Yes/yes	No	No	10	Non 16/18	33	LSIL	Normal	Negative	13	Negative	Negative
4	36	No	Bu/Flu	No/yes	No	No	12	16	16,42,54,82	LSIL	Normal	Negative	34	Negative	Negative
5	46	LSIL	Bu/Cy	No/yes	Chronic	Treatment with tacrolimus	37	Non 16/18	51	LSIL	Normal	Negative	55	Non 16/18 (51)	LSIL
6	31	No	Bu/Flu/Thi/ Cy	No/no	No	Prophylaxis with tacrolimus	16	Non 16/18	31,42,44,53,54,73	HSIL	Abnormal	HSIL[a]	30	Negative	Negative
7	48	No	Bu/Cy	No/no	Chronic	No	5	18	18	HSIL	Normal	HSIL[b]	56	Negative	Negative
8	28	No	Cy/TBI	No/no	No	No	11	Non 16/18	31	Negative	Normal	No	Death during follow-up		
9	26	LSIL	Flu/Cy/TBI	No/no	No	No	27	Non 16/18	31	Negative	Normal	No	41	Non 16/18 (31)	LSIL
10	40	No	Flu/Cy/TBI	No/no	No	Prophylaxis with cyclosporine	4	Non 16/18	52	Negative	Normal	Negative	30	Non 16/18 (52)	Negative

[a]Vaginal HSIL.

[b]HSIL diagnosed in the endocervical curettage.

allo-HSCT, allogeneic hematopoietic stem cell transplantation; Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; GvHD, graft-vs-host disease; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; Mel, melphalan; Pap, Papanicolaou; TBI, total body irradiation; Thi, thiotepa.

had not undergone pre-HSCT gynecological evaluations in the previous 5 y. Thus, previous CC screening results based on Pap smear with no HPV testing were available in only 34 women (48.6%). Of the 34 women, 27 (79.4%) had negative Pap smears, 3 (8.8%) showed LSILs, and 4 (11.8%) showed HSILs. The 3 patients with a previous diagnosis of LSILs showed LSILs in the gynecological evaluation: 1 at the initial screening and 2 during the follow-up; all presented high-risk HPV types other than 16/18. Of the 4 women with a previous diagnosis of HSILs, 1 showed LSIL in the first evaluation, 1 showed an HSIL diagnosed during the follow-up, 1 showed treatment-related atypia, and 1 woman presented negative screening tests. Fourteen of 29 women (48.3%) with treatment-related reactive atypia had undergone previous CC screening. In all but one of them, the previous cytology was negative. Only 1 patient had undergone cervical conization due to HSIL 11 y before allo-HSCT but presented normal Pap smears after the treatment.

HPV Vaccination

Fourteen of 70 women (20%) received the HPV vaccine after allo-HSCT. The 6 women younger than 26 y had been previously vaccinated in accordance with the National Vaccination Program.[20] The 6 women were revaccinated after allo-HSCT. Six of the 14 vaccinated women had a positive HPV test result in the first gynecological evaluation: 2 showed a negative HPV test after vaccination, 1 died, 1 was lost to follow-up, and 2 continued being HPV positive after vaccination.

Follow-up Results

The mean follow-up was 51.6 ± 33.3 mo. During follow-up, 3 of 70 women included in the study died as a result of disease progression (1 died 24 mo after allo-HSCT and 2 died 16 mo after allo-HSCT).

Among the 10 women with a positive HPV test result at the initial evaluation, 1 died due to the progression of the hematologic disease and 1 was lost to follow-up. Two women spontaneously cleared the HPV infection, 2 cleared the infection after treatment, and 4 showed persistent HPV infection at the past evaluation.

Among the 60 women with a negative HPV test at the initial gynecological evaluation (including the 29 with treatment-related atypia), 2 died of progression of the hematologic disease. Five of 58 patients (8.6%) alive became HPV positive during follow-up: 1 presented HPV16 and 4 had high-risk HPV types other than 16/18. The Pap smear showed an LSIL in 4 of these women, which was histologically confirmed in 3. The Pap smear showed HSIL in 1 woman and the lesion was histologically confirmed. Table 3 shows the main clinical and pathological features of the women who became HPV-positive during follow-up. The mean time from the first control with a negative HPV test to incident HPV infection and an abnormal Pap smear was 30.4 ± 13.1 mo (range, 14–48). The mean time from allo-HSCT to the detection of the incident HPV infection was 39.4 ± 9.2 mo (range, 19–68).

The cumulative prevalence of HPV infection was 21.4% (15/70), with 10 women being HPV positive at the initial evaluation and 5 women becoming positive during follow-up.

TABLE 3.

Patients who underwent allo-HSCT and had a normal or reactive cytological result at the first control evaluation and developed abnormal cytology results during follow-up

Case	Age, y	Previous screening	Months after allo-HSCT (first evaluation)	Active GvHD	Immunosuppressive drugs	HPV testing (first evaluation)	Cytology (first evaluation)	Months after allo-HSCT to HPV positive testing	HPV testing (follow-up)		Cytology (follow-up)	Colposcopy	Biopsy
									Cobas	Allplex			
11	62	No	5	No	Prophylaxis with tacrolimus	Negative	Reactive atypia	19	Non 16/18	33	LSIL	Normal	Negative
12	43	No	11	Acute	Treatment with tacrolimus/prednisone	Negative	Reactive atypia	47	Non 16/18	54	LSIL	Abnormal[a]	Vaginal LSIL
13	39	HSIL	6	No	Prophylaxis with tacrolimus	Negative	Negative	38	Non 16/18	56	HSIL	Abnormal	Cervical HSIL
14	23	LSIL	3	No	No	Negative	Negative	25	Non 16/18	31	LSIL	Abnormal	Cervical LSIL
15	25	No	20	Chronic	Treatment with prednisone/extracorporeal photopheresis	Negative	Negative	68	16	16	LSIL	Abnormal	Cervical LSIL

[a]Vaginal lesion.

allo-HSCT, allogeneic hematopoietic stem cell transplantation; GvHD, graft-vs-host disease; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions.

All Pap smear reactive abnormalities spontaneously disappeared during follow-up, but 2 patients presented an incident HPV infection (HPV other than 16/18) during follow-up and developed LSILs. The mean time from the first control with reactive atypia to regression of the cytological abnormality was 15.2 ± 11.6 mo (range, 5–62). The mean time from allo-HSCT to spontaneous regression of the cytological abnormality was 25.2 ± 13.2 mo (range, 13–74).

DISCUSSION

Although female recipients of allo-HSCT are a high-risk group for HPV infection and CC,[4,6,32] very few studies have addressed the screening of CC in these women. In the present study, we analyzed the results of a program of gynecological evaluation and CC screening, which included HPV testing and genotyping as well as Pap smear in a series of 70 female recipients of allo-HSCT.

The prevalence of Pap smear abnormalities identified in our series (51.4%) is in keeping with the percentages of abnormal Pap smear results ranging from 11.1% to 68.5% in previous studies.[6-8,33] However, most of the previous studies did not include data on HPV testing or histological confirmation, and thus, the prevalence of Pap smear abnormalities reported might be obscured by the inclusion of reactive atypia related to treatment together with HPV-associated atypia.

The most remarkable result of our study was the high cumulative prevalence of HPV infection (21.4%; 15/70), with 10 women being HPV positive in the initial evaluation and 5 additional women becoming positive during follow-up. This prevalence is higher than the prevalence of HPV of 5%–10% reported in women undergoing CC screening in Catalonia, the region of Spain where the present study was conducted.[34-36] Only 2 previous studies have reported the HPV prevalence in female recipients of allo-HSCT. Both studies described a high prevalence of HPV (18.3% and 27.9%, respectively), which is in keeping with our series. Neither of the 2 previous studies included HPV genotyping.[5,8] HPV genotyping in our study showed a high prevalence of high-risk HPV types other than 16/18 (12/15; 80.0% of the HPV infections). Most of the HPV-positive women had mild cytological abnormalities (negative or LSIL result), but 3 patients had HSIL, which was histologically confirmed in all cases. A remarkable finding of our study was the relatively high proportion of incident HPV infections detected during the follow-up in patients who were initially negative. Thus, our study not only confirms the importance of establishing gynecological care as part of the multifaceted posttransplantation care program but also shows that gynecological evaluations, especially those focused on CC prevention, should be maintained over time.

A striking result of our study was the high prevalence of reactive atypia closely mimicking LSIL and HSIL related to the conditioning regimen identified in these patients (41.4%). In all these cases, HPV testing provided key information for achieving an accurate diagnosis, avoiding overdiagnosis of HPV-associated lesions and patient overtreatment. Indeed, a high-risk HPV-negative result was a defining criterion to establish the diagnosis of treatment-related reactive atypia in this study.

Remarkably, although low-risk HPV types have shown to be responsible for a small percentage of LSIL cytological abnormalities (around 15%),[35,36] and hypothetically could be involved in the reactive changes identified in these patients, all but 1 patient with cytological abnormalities associated with the treatment had a negative test result for low-risk HPV types, which allows excluding the role of these viruses in the development of these cytological changes. Interestingly, although these abnormalities were very frequent in the first evaluation (mean time 9.6 ± 7.4 mo after allo-HSCT), they all regressed during follow-up. These data are in keeping with the results reported by other investigators.[6,17,18] Similar changes have been described in patients undergoing radiation therapy for CC,[37] but none of these patients have received radiation.

Only 4 previous studies, including the 1 published by our group, have evaluated reactive atypia related to treatment in female recipients of allo-HSCT. In the previous studies, the percentage of women showing these changes in the Pap smear ranged from 5.5%[6] to 11%,[18] which is much lower than the prevalence observed in the present study (41.4%). This lower prevalence might be due to misclassifying some of these abnormalities, which could have been classified as HPV-associated atypia, as the final diagnosis in these studies was based purely on morphological criteria. Thus, HPV testing, which was not reported in most of the previous series, is key to differentiating HPV-associated and treatment-related atypia.

In a previous study by our group evaluating reactive atypia related to treatment, busulfan was the agent most commonly associated with these abnormalities.[16] In the present series, 72.9% of the women (51/70) undergoing allo-HSCT had received busulfan, and among these, 54.9% (28/51) showed treatment-related atypia in the Pap smear simulating either HSILs or LSILs. Indeed, only 1 of the women with treatment-related atypia had not received busulfan (she received fludarabine instead). Although the relationship between busulfan and reactive atypia related to treatment seems to be strong, the differences in terms of the percentage of women receiving any of these drugs who developed atypia were not statistically significant. In addition, many of these women received >1 alkylating drug, resulting in serious difficulties in attributing these changes to a single drug.

In our study, there were no differences in the prevalence of HPV infection between women with and without GvHD (14.3% versus 14.3%). Neither were there any differences in terms of women showing treatment-related reactive atypia between those presenting or not GvHD (48.3% versus 51.2%). These results are in contrast with other studies reporting a higher risk of HPV-related lesions in women with GvHD.[4,5,7,8,18,33]

This study has several strengths. First, a thorough gynecological assessment was offered to all female recipients of allo-HSCT. Second, HPV testing, Pap smear, and a complete evaluation of the lower genital was performed in all the women. Finally, our study included a long follow-up period, which allowed the detection of incident HPV infections and the monitoring of reactive atypia related to treatment. There are, nevertheless, some weaknesses, such as the low number of patients included, although it should be emphasized

that the present series is one of the largest series of women undergoing allo-HSCT reported in the literature.[38] Another limitation is the low percentage of women with data available on previous CC screening results (<50%), which is similar to the percentage observed in other series.[38] These low screening rates have been attributed to the multiple medical problems associated with hematologic neoplasia, the side effects of the immunosuppressive HSCT therapy, and the higher likelihood of cytopenia, including thrombocytopenia, which could preclude cervical procedures.

In conclusion, female recipients of allo-HSCT are a high-risk population for HPV infection and (pre)cancer. Gynecological evaluations should be maintained over time, as a significant proportion of these women may become HPV-positive during follow-up. Reactive benign, atypical changes related to treatment, which mimic HPV-associated lesions, are a frequent finding in these women. HPV testing is a key tool for the evaluation of these patients, as it allows for identifying women at risk and excluding cytological mimickers.

REFERENCES

1. Inamoto Y, Shah NN, Savani BN, et al. Secondary solid cancer screening following hematopoietic cell transplantation. *Bone Marrow Transplant*. 2015;50:1013–1023.
2. Division of Cancer Prevention and Control, Centers for Disease Control and Prevention. Human papillomavirus (HPV)-associated cancers. Cancers Associated with Human Papillomavirus | U.S. Cancer Statistics | CDC. Available at https://www.cdc.gov/united-states-cancer-statistics/publications/hpv-associated-cancers.html. Accessed October 13, 2023.
3. Maglennon GA, McIntosh PB, Doorbar J. Immunosuppression facilitates the reactivation of latent papillomavirus infections. *J Virol*. 2014;88:710–716.
4. Sasadeusz J, Kelly H, Szer J, et al. Abnormal cervical cytology in bone marrow transplant recipients. *Bone Marrow Transplant*. 2001;28:393–397.
5. Wang Y, Brinch L, Jebsen P, et al. A clinical study of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2012;18:747–753.
6. Negri G, Herz M, Deola S, et al. Abnormal cervical cytology after allogeneic bone marrow transplantation. *Am J Clin Pathol*. 2014;142:222–226.
7. Savani BN, Stratton P, Shenoy A, et al. Increased risk of cervical dysplasia in long-term survivors of allogeneic stem cell transplantation—implications for screening and HPV vaccination. *Biol Blood Marrow Transplant*. 2008;14:1072–1075.
8. Shanis D, Anandi P, Grant C, et al. Risks factors and timing of genital human papillomavirus (HPV) infection in female stem cell transplant survivors: a longitudinal study. *Bone Marrow Transplant*. 2018;53:78–83.
9. Doorbar J. Latent papillomavirus infections and their regulation. *Curr Opin Virol*. 2013;3:416–421.
10. Tanweer MS, Aljurf M, Savani BN, et al. Lower genital tract precancer and cancer in hematopoietic cell transplant survivors and the role of HPV: a systematic review and future perspectives. *Clin Hematol Int*. 2019;1:142–153.
11. Savani BN, Griffith ML, Jagasia S, et al. How I treat late effects in adults after allogeneic stem cell transplantation. *Blood*. 2011;117:3002–3009.
12. Majhail NS, Rizzo JD, Lee SJ, et al; Center for International Blood and Marrow Transplant Research. Recommended screening and preventive practices for long-term survivors after hematopoietic cell transplantation. *Bone Marrow Transplant*. 2012;47:337–341.
13. Iglesias Álvarez M, Ruíz Marzal F, Menor Almagro D, et al. Boletín de la Asociación Española de Patología Cervical y Colposcopia. Available at http://www.aepcc.org/wp-content/uploads/2016/01/Boletin-AEPCC-Septiembre-2015.pdf. Accessed October 13, 2023.
14. Dyer G, Larsen SR, Gilroy N, et al. Adherence to cancer screening guidelines in Australian survivors of allogeneic blood and marrow transplantation (BMT). *Cancer Med*. 2016;5:1702–1716.
15. Bishop MM, Lee SJ, Beaumont JL, et al. The preventive health behaviors of long-term survivors of cancer and hematopoietic stem cell transplantation compared with matched controls. *Biol Blood Marrow Transplant*. 2010;16:207–214.
16. Saco A, Alòs S, Esteve R, et al. Atypical cytological changes mimicking SIL of the uterine cervix in allogenic hematopoietic stem cell transplantation recipients treated with busulfan. *Cancer Cytopathol*. 2019;127:399–406.
17. Koss LG, Melamed MR, Mayer K. The effect of busulfan on human epithelia. *Am J Clin Pathol*. 1965;44:385–397.
18. Yu SC, Huang HH, Li CC, et al. Cervical Papanicolaou smears in hematopoietic stem cell transplant recipients: high prevalence of therapy-related atypia during the acute phase. *Biol Blood Marrow Transplant*. 2017;23:1367–1373.
19. Solomon D, Davey D, Kurman R, et al; Forum Group Members. The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA*. 2002;287:2114–2119.
20. Alameda F, Daniel Andía D, Xavier Castellsagué X, et al. AEPCC-Guía: prevención Del Cáncer de Cuello de Útero; 2015. Available at https://www.aepcc.org/wp-content/uploads/2015/05/AEPCC_revista01.pdf. Accessed October 13, 2023.
21. Bornstein J, Bentley J, Bösze P, et al. 2011 Colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. *Obstet Gynecol*. 2012;120:166–172.
22. Tatti S, Bornstein J, Prendiville W. Colposcopy: a global perspective. *Obstet Gynecol Clin North Am*. 2013;40:235–250.
23. van der Marel J, van Baars R, Rodriguez A, et al. The increased detection of cervical intraepithelial neoplasia when using a second biopsy at colposcopy. *Gynecol Oncol*. 2014;135:201–207.
24. Bergeron C, Ordi J, Schmidt D, et al; European CINtec Histology Study Group. Conjunctive p16INK4a testing significantly increases accuracy in diagnosing high-grade cervical intraepithelial neoplasia. *Am J Clin Pathol*. 2010;133:395–406.
25. WHO Classification of Tumours Editorial Board. Classification of tumours of female reproductive organs. *WHO/IARC Classification of Tumours*. Vol 4, 5th ed; 2020.
26. Darragh TM, Colgan TJ, Thomas Cox J, et al; Members of the LAST Project Work Groups. The Lower Anogenital Squamous Terminology Standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. *Int J Gynecol Pathol*. 2013;32:76–115.
27. Harris AC, Young R, Devine S, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai Acute GvHD International Consortium. *Biol Blood Marrow Transplant*. 2016;22:4–10.
28. Frey Tirri B, Häusermann P, Bertz H, et al. Clinical guidelines for gynecologic care after hematopoietic SCT. Report from the international consensus project on clinical practice in chronic GVHD. *Bone Marrow Transplant*. 2015;50:3–9.
29. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant*. 2005;11:945–956.
30. DeLord C, Treleaven J, Shepherd J, et al. Vaginal stenosis following allogeneic bone marrow transplantation for acute myeloid leukaemia. *Bone Marrow Transplant*. 1999;23:523–525.
31. Protocol de Detecció Precoç Del Càncer de Coll Uterí a Catalunya. WHO Classification of Tumours Editorial Board. Available at https://scientiasalut.gencat.cat/bitstream/handle/11351/10538/protocol_deteccio_preco%C3%A7_cancer_coll_uteri_catalunya_2023.pdf?sequence=1&isAllowed=y. Accessed February 21, 2024.
32. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. *Lancet*. 2007;370:890–907.
33. Klasa L, Sadowska-Klasa A, Piekarska A, et al. The management of gynecological complications in long-term survivors after allogeneic hematopoietic cell transplantation—a single-center real-life experience. *Ann Hematol*. 2020;99:1361–1368.
34. Bruni L, Diaz M, Castellsagué X, et al. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *J Infect Dis*. 2010;202:1789–1799.
35. Ibáñez R, Roura E, Monfil L, et al. Long-term protection of HPV test in women at risk of cervical cancer. *PLoS One*. 2020;15:e0237988.
36. Lloveras B, Gomez S, Alameda F, et al. HPV testing by Cobas HPV test in a population from Catalonia. *PLoS One*. 2013;8:e58153.
37. Rintala MA, Rantanen VT, Salmi TA, et al. PAP smear after radiation therapy for cervical carcinoma. *Anticancer Res*. 1997;17:3747–3750.
38. Hwang JP, Ahmed S, Ariza-Heredia EJ, et al. Low rate of cervical cancer screening among women with hematologic malignancies after stem cell transplant. *Biol Blood Marrow Transplant*. 2018;24:1094–1098.