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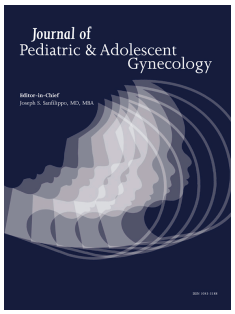


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# Patterns of Genital Examination and Vulvovaginal Graft-Versus-Host Disease in a Pediatric Post-Hematopoietic Stem Cell Transplant Population

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- Liang CS, Allen SM, Pascoe KF, Chesnokova AE, Childress KJ, Dietrich JE: Prevalence and surveillance of vulvovaginal graft versus host disease after hematopoietic stem cell transplant in the pediatric population: an analysis of 84 patients. J Pediatr Adolesc Gynecol 2019; 32:2. DOI: https://doi.org/10.1016/j.jpag.2019.02.096

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ABSTRACT

Study Objective:
To determine vulvovaginal (vv) GVHD incidence among pediatric patients who are post-hematopoietic stem cell transplant (HSCT) and who already have GVHD involving any organ system and characterize patterns of genital examination and referral to pediatric and adolescent gynecology (PAG) in the post-HSCT population

Design:
Retrospective chart review

Setting:
Large tertiary children's hospital in Texas

Participants:
86 post-HSCT female patients ≤21 years old with GVHD involving any organ system

Interventions:
None

Main Outcome Measures:
vvGVHD among post-HSCT children, referrals to PAG, genital examinations documented by any clinician

Results:
86 patients met inclusion criteria. Most HSCTs were bone marrow transplants, typically for leukemia. Median ages of indication diagnosis and HSCT were 5.1 and 7.5 years, respectively. Median time from HSCT to first GVHD diagnosis (e.g. skin, intestine) was 96 days. Nearly all patients had at least 1 genital exam documented in the first 2 years post-HSCT, with a median of 17 exams. 28 patients were seen by PAG post-HSCT, with 7 of these patients seen within the first 2 years post-HSCT. Four symptomatic patients were diagnosed with vvGVHD. Median time from HSCT to vvGVHD was 398 days.

Conclusion:
The small number of vvGVHD cases in our study population is likely due to lack of symptom reporting from patients and families and difficulty with vvGVHD diagnosis. Further training for non-PAG physicians, including pediatricians and oncologists, in identifying and managing vvGVHD may prevent delayed diagnosis and severe sequelae. Earlier referral to PAG or a gynecologist versed in post-HSCT survivorship is also recommended.

Keywords: Graft vs Host Disease; Hematopoietic Stem Cell Transplantation; Vulvar diseases; Cancer survivors; Transplant recipients

## Introduction

Chronic graft-versus-host disease (GVHD) is the most common cause of poor quality of life following hematopoietic stem cell transplantation (HSCT).[1] As HSCT continues to improve survivorship among patients with hematopoietic malignancies and nonmalignant conditions of the bone marrow and immune system, there is increased focus on identification and treatment of long-term sequelae, namely GVHD. [2,3] Areas most commonly affected are the skin, oral mucosa, eyes, liver, and intestine.[4,5]

Chronic GVHD is the most common cause of vulvovaginal symptoms after HSCT in adult and pediatric females.[6] The reported incidence ranges from 3% to 49%, but the true incidence has not been established.[7–9] A 2019 case series by Cizek and colleagues found that 5.9% of all post-HSCT female children followed in one pediatric hospital system developed vulvovaginal GVHD (vvGVHD).[10] Symptoms of vvGVHD in general include: vulvar irritation, burning, dysuria, and dyspareunia.[6,11] Clinical exam findings from the adult literature may range from vulvar erythema, lichen planus-like features (including, but not limited to, reticular white lines on genital mucosa)[12], tenderness to palpation of the Bartholin's or Skene's glands openings, labial adhesions/agglutination, erosions, and fissures to introital stenosis and vaginal synechiae.[3,6] Among pediatric patients with vvGVHD, the most common exam findings are vulvar adhesions/agglutination, vulvar atrophy, labial erosions, and vestibular pain on exam.[10] Severity scoring for vvGVHD has been detailed by Stratton et al.[6] Cizek et al.[10] have suggested severity scoring specific to vulvar GVHD in the pediatric population. From the wider literature, some including pediatric patients, isolated vvGVHD is rare; typically it occurs in the context of current or past GVHD involving another organ system, most commonly skin, oral mucosa, or eyes.[6,8] While systemic therapy for chronic GVHD has not been found to prevent or effectively treat vvGVHD, localized treatment with topical steroids, estrogen replacement and management with vaginal dilators and/or surgical intervention have been found to be effective in treating vvGVHD in the adult population.[6,9] Concomitant diagnosis of primary ovarian insufficiency (POI)

also influences the treatment of vvGVHD, with the addition of systemic and topical hormone replacement therapy to the regimen.[10]

While there are case reports on vvGVHD among pediatric, adolescent and young adult females, most describe the advanced cases needing surgical management, such as vaginal stenosis, hematocolpos, and labial fusion.[13–16] While there are screening recommendations for the adult post-HSCT population (gynecologic examination recommended annually, and every three months in the setting of severe GVHD involving any organ system)[17], there are currently no vvGVHD screening guidelines for post-HSCT children. Furthermore, to our knowledge, there are no studies detailing vulvovaginal complaints and genital examination patterns of post-HSCT children who already have a diagnosis of GVHD of any organ system, and are thus at high risk of vvGVHD.

In this study, we characterize post-HSCT female pediatric patients at a large pediatric hospital who had a clinical history of GVHD involving any organ system, with a focus on: 1) referrals to pediatric and adolescent gynecology (PAG), 2) frequency of genital exams documented by any clinician and those performed by PAG within two years of HSCT, 3) incidence of vvGVHD among post-HSCT children with a history of GVHD of any organ system, and 4) clinical histories of patients diagnosed with vvGVHD.

## Materials and Methods

*Setting and participants.* We conducted a retrospective chart review of female patients ≤21 years of age who had a history of HSCT, any subsequent GVHD diagnosis, and were seen at Texas Children's Hospital (TCH) between 2007 and 2018. Inclusion criteria were as follows: female, history of HSCT, ≤21 years of age at the time they were seen at TCH, and clinical diagnosis of GVHD involving any organ system. We included patients who had HSCT for malignant and nonmalignant conditions. We included patients who were deceased at the time of data collection. While all patients had at least one HSCT that took place at TCH, some

underwent previous HSCTs at other institutions. Patients were excluded if they underwent solely autologous HSCT, as these patients are not at risk for GVHD.

*Data sources and management.* Data collected included basic demographics, clinical characteristics of indication disease necessitating HSCT, HSCT type, human leukocyte antigen (HLA)-matching, clinical and histological characteristics of GVHD diagnosis, clinical data from inpatient consults or office visits with PAG, and documentation and number of genital exams by any clinician involved in the patient's care in the TCH system. Female pediatric patients who underwent HSCT, and developed GVHD of any organ system were identified using ICD10 diagnosis codes (e.g. D89.81 for GVHD). While there is no ICD10 code for vvGVHD, the following codes were also used to identify any possible cases of isolated vvGVHD: vulvovaginal discomfort (N94.89), disease (N90.89), dryness (N94.89), itching/pruritus (L29.2), pain (N94.89), vulvovaginitis/vaginitis (N76.0), and vulvovaginitis associated with another disease (N77.1). Data were entered into an Excel spreadsheet and stored in a HIPAA-compliant cloud software program through Baylor College of Medicine. The project received approval from the Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals.

*Variables and diagnostic criteria.* Patients who received a bone marrow transplant (BMT) or peripheral blood stem cell transplant (PBSCT) fell into one of several categories based on their relation to the donor and the degree of HLA matching at five loci (A, B, C, DR, DQ, on both sets of chromosomes, for a total of 10): matched unrelated donor (10/10 match), matched related donor (i.e. sibling, 10/10 match), haploidentical donor (i.e. parent, 5/10 match), mismatched unrelated donor (9/10 match), or mismatched related donor (9/10 match). Patients who received cord blood transplants were categorized based on their relation to donor and the degree of HLA matching at three loci (A, B, DR, on both sets of chromosomes, for a total of 6): matched unrelated donor (6/6 match), matched related donor (i.e. sibling, 6/6 match), mismatched unrelated donor (4/6 or 5/6 match), or mismatched related donor (4/6 or 5/6 match).

Patients were also categorized by the organ systems where they developed GVHD, including skin, lung, liver, eyes, intestine, oral mucosa, and vulva and/or vagina.

Furthermore, data were collected on the frequency of genital exams by any clinician in the two years following HSCT by reviewing the physical exam in each progress note post-transplant. The range of what was considered a genital exam by a clinician was wide and varied, including anything from Tanner staging to a vulvovaginal exam under anesthesia and/or vaginoscopy. Genital exams performed by non-PAG clinicians as well as the number performed by PAG clinicians in the first two years following a patient's first HSCT were included.

Data were also collected on whether patients were evaluated by PAG, reason for referral and if any gynecologic exam was performed by PAG. Both clinic visits and inpatient consults were considered evaluations by PAG.

Lastly, vvGVHD was defined as vulvar erythema, lichen planus-like features, tenderness to palpation of the Bartholin's or Skene's glands openings, labial adhesions/agglutination, erosions, fissures, introital stenosis and/or vaginal synechiae.[6,11] As vvGVHD is a clinical diagnosis, biopsy was not necessary. Clinical history of events leading to vvGVHD diagnoses were documented.

*Statistical methods.* Descriptive statistics including median, interquartile range (IQR) for continuous variables, and frequency and proportion for categorical variables were calculated. $X^2$ test was utilized for categorical variables and Wilcoxon rank-sum test for continuous variables. Data were analyzed using Stata 15 (StataCorp, College Station, TX).

## Results

*Demographics*

Of the approximately 380 female patients who underwent HSCT at TCH during the study period, 86 met criteria for inclusion in our study. More than half (55%) were Latina, one-third were white/non-Latina, and the remaining 12% were Black, Asian or other. Half (49%) of

patients had insurance coverage through Medicaid or other state-funded programs. Median age at diagnosis for indication disease was 5.1 years (IQR: 1.6—10.9), but children who were diagnosed with a malignant condition tended to be older at diagnosis than children who were diagnosed with a nonmalignant condition (7.4 years vs. 1.1 years, p<0.001).

*HSCT indications and characteristics*

By definition, all patients in the study underwent HSCT: nearly two-thirds (64%) for a malignant diagnosis, such as leukemia or lymphoma, the remaining 36% for a nonmalignant diagnosis, such as bone marrow failure or an immune deficiency disease (Table 1).

Patients underwent HSCT at a median of 7.5 years of age (IQR: 3.7—12.7). Median time from indication diagnosis to HSCT was 247 days (IQR: 121—798); this did not differ between those who had a malignant indication diagnosis and those with a nonmalignant diagnosis (p=0.500). Sixty-three percent of patients received a transplant from an HLA-matched source (either related, 29%, or unrelated, 35%). The remaining patients received a transplant from sources that were either haploidentical (16%), such as a parent, or a mismatched donor, either related (3%) or unrelated (16%). While most children in the study had a BMT (Table 2), those with a malignant diagnosis were more likely to have had a PBSCT (23% vs. 6%) whereas those with a nonmalignant diagnosis were more likely to have had an umbilical cord blood transplant (19% vs 7%) (p=0.05).

*GVHD Characteristics*

Most (n=74, 86%) patients received one or more medications for GVHD prophylaxis. The most common agents included in prophylactic regimen were tacrolimus (49%), cyclosporine (34%), and methotrexate (30%) (Table 3). Only 6% received no GVHD prophylaxis and an additional 8% had missing information for GVHD prophylaxis.

By definition, all patients in our study went on to develop GVHD in one or more organ systems (Table 4). Median time from transplant to first GVHD diagnosis was 96 days (IQR: 35—210). For three patients the date of first diagnosis of GVHD could not be determined, due to

conflicting dates in the chart. Their GVHD diagnosis dates were thus said to be missing. Skin was the most common site of GVHD (87%), followed by intestine (26%), liver (23%), and oral mucosa (20%). Over half of those who had skin GVHD had no evidence of GVHD at other sites. This is in contrast to every other site of GVHD, which were much more likely to appear in the setting of GVHD at another site, most commonly skin. No cases of ocular or vvGVHD appeared in isolation. Nearly half (44%) of patients had evidence of GVHD in more than one organ system.

*Genital exams and Pediatric and Adolescent Gynecology evaluations*

In the first two years post-HSCT, 86% of patients had at least one genital exam documented in the medical record. Seven patients were seen by PAG within the first two years post-HSCT and underwent a median of three exams (IQR: 2—4). Additionally, most patients (n=76, 85%) had a genital exam by a non-PAG clinician in the first two years post-HSCT, with a median of 17 exams (IQR: 6—33). Of the 1712 genital exams performed in the first two years following transplant, 1% were performed by PAG clinicians.

While only seven patients saw PAG within the first two years post-HSCT, an additional 21 were seen by PAG later post-HSCT (median number of PAG visits/consultations: 3, IQR: 2—7). Three more patients were referred to PAG but did not keep the clinic appointment. Another seven patients were referred to outside general gynecologists instead of PAG, three of which were seen. For the three outside gynecologist visits, little information was available. In what was available, there was no mention of a concern for GVHD or vulvar complaint consistent with vvGVHD. The most common reasons for referral to a gynecology provider included diagnosis of or concern for POI, and abnormal uterine bleeding. Four patients were referred to PAG for a vulvovaginal problem, including vulvar lesions, vulvovaginitis, vaginal atresia/hematocolpos, and concern for vvGVHD (Table 5).

Relatively few (n=13, 15%) patients were menarchal at the time of their indication diagnosis. Median age at menarche in the study was 13 years (IQR: 12—15). Of note, 13

patients passed away before menarche and another 26 had not reached menarche at the time of data collection. The prevalence of any diagnosis of POI was 43%.

*Vulvovaginal GVHD Cases*

There were four cases of vvGVHD in this group of patients (5% incidence among patients who already had a diagnosis of GVHD, 1.2% incidence among all female pediatric patients post-HSCT). Each case is described and graded according to both the Adult and Pediatric vvGVHD scales in Table 6. [6,10] Two of four patients were older adolescents (18-19 years of age), and the remaining two were prepubertal when they developed symptoms of vvGVHD. Three of four cases were seen and diagnosed by PAG, the remaining case was diagnosed and managed by dermatology. One case had a nonmalignant indication diagnosis (Griscelli Syndrome with Hemophagocytic Lymphohistiocytosis), while the remaining three had indication diagnosis of AML. Two of the four patients with vvGHVD had undergone BMT; one had undergone PBSCT; one underwent both BMT and PBSCT. Only one of the three had a vaginal exam; the extent of vaginal disease is unknown in the other cases. Case 1 was diagnosed after several treatments for vulvovaginitis. Ultimately, she was found to have scarring consistent with vvGVHD. Case 2 had vulvar pain and a diffuse rash that included the vulva, was diagnosed with vvGVHD by dermatology, who also initiated treatment. Case 3 was an adolescent who had vulvar GVHD in the setting of GVHD of the skin. Case 4 developed significant labial agglutination secondary to vvGVHD. She was treated with estrogen creams with some improvement, but ultimately needed surgical intervention. Timelines of indication diagnosis, transplant and development of GVHD for each case of vvGVHD are illustrated in Figures 1a-d.

## Discussion

In this group of 86 children who received HSCT for malignant or nonmalignant conditions and went on to develop GVHD involving any organ system, incidence of vvGVHD was low (5%).

Most patients had at least one genital exam documented during their first two years post-HSCT. One-third saw PAG at any point post-HSCT, typically for POI concerns, abnormal bleeding, or vulvovaginal complaints. vvGVHD cases ranged from those that were mild and treated successfully with topical creams to one case that required surgical management.

While non-PAG physicians certainly can identify genital abnormalities in this population, it has been well established that, when examining prepubertal girls, many providers, including pediatricians, are unable to distinguish between normal anatomical variants and genital pathology.[18–20] Pediatric residency programs have instituted more formal training in PAG over the past two decades,[21] however, the degree of exposure to PAG training varies. The North American Society for Pediatric and Adolescent Gynecology has created a variety of curricula for trainees from centers with limited formal PAG experience.[22] Wider use of such materials by providers caring for pediatric and adolescent post-HSCT patients could minimize underdiagnosis of vvGVHD. There is also a need to decrease provider bias in asking about vulvovaginal symptoms in this young population. Considering the above points and that children and families may underreport genital symptoms, there is a significant risk of underdiagnosis of this debilitating condition.[23]

In addition to poor detection of genital pathology in female pediatric patients overall, there is further evidence that, among children younger than ten years, girls have historically received genital examinations almost half as frequently as boys, with only a third of girls ages 5-10 years being examined by their primary care provider.[24] A more recent study from the child abuse literature found that 90% of pediatric chief residents examined the genitalia of a prepubescent girl in at least half of annual visits.[18] This demonstrates an improvement, but the American Academy of Pediatrics recommends “at a minimum, examination of the external genitalia should be included as part of the annual comprehensive physical examination of children and adolescents of all ages”.[25] In this study of female pediatric patients with a history of

HSCT and GVHD, most patients had at least one genital exam documented in the first two years post-HSCT.

Only 1% of genital exams these patients received in the first two years post-HSCT were performed by PAG clinicians. While 33% of patients were seen in PAG clinic or were evaluated by PAG while inpatient at any point post-HSCT, an additional three were referred, but did not attend outpatient clinic appointments. Three additional patients were seen by general gynecology, although it is unclear why they were not instead seen by PAG. Since one of the most common reasons for referral to PAG or general gynecology was POI, a concern with mainly long-term health implications such as infertility or bone loss, it is plausible that attending appointments addressing more acute matters was priority for these families. While the prevalence of POI in the post-HSCT population is relatively high due to gonadotoxic therapies, it is crucial to note that the symptoms of vvGVHD can overlap with those of the reduced estrogen state in POI, including vulvovaginal pain and irritation.[9]

Four (5%) patients were diagnosed with vvGVHD. As only one-third of patients were seen by PAG at any point post-HSCT, it is likely that this is an underestimate of vvGVHD in this population. Five of the patients who were seen by PAG presented for a vulvovaginal complaint. One was an adolescent who presented for vulvovaginal lesions found on biopsy to be lichen simplex chronicus and an HPV-associated condyloma. While this patient was not found to have vvGVHD, her early HPV disease is important to note, as reactivation of HPV and other viruses is common after transplant, given these patients typically have a long period of immunosuppression. A recent case report highlights the dramatic presentation and complicated management of concomitant severe vvGVHD and florid HPV disease in an adult female who had undergone HSCT.[26] Another patient who presented to PAG for a vulvovaginal complaint was determined to have vaginal atresia and subsequent hematocolpos. Although hematocolpos due to vulvovaginal adhesions and vaginal obstruction can be a severe manifestation of vvGVHD,[6,12,13] the findings in this patient were determined to be a congenital lower reproductive

tract anomaly, not attributed to vvGVHD. Lastly, one patient who was ultimately diagnosed with vvGVHD (Case 1) was evaluated by PAG multiple times for vulvovaginitis prior to her vvGVHD diagnosis. Her initial presentation is important because young girls are especially vulnerable to vulvovaginal irritation due to non-estrogenized genital tissue, improper wiping and frequent contact with irritants such as bubble bath, soaps and wet wipes.[23,27,28] While this patient's treatment with topical corticosteroid creams improved her symptoms, had her disease gone undiagnosed, she may have developed more severe structural vvGVHD manifestations such as vaginal stenosis. This case highlights the need for vulvovaginitis in a pre-pubertal post-HSCT child to prompt evaluation for vvGVHD.

While all four cases in our study were symptomatic, a recent case series of female children developing vvGVHD post-HSCT found that a higher proportion of pediatric patients in the series were asymptomatic compared to women in the adult literature.[10] This potential for insidious onset of disease emphasizes the need for regular genital exams for children and adolescents post-HSCT.

All cases of vvGVHD occurred in patients with a history of or current GVHD of the skin elsewhere on the body; three of the four cases occurred in the setting of past or current intestinal GVHD. This is consistent with the adult literature: a cohort of post-HSCT adult women found that women with vvGVHD had a high rate of chronic GVHD in other skin and mucosal surfaces.[6] This also suggests that, while the vulva and/or vagina are rarely the initial site of GVHD, the likelihood of vulvovaginal involvement increases when the disease is present on other skin or mucosal areas.

The median time from transplant to development of vvGVHD in our study was 398 days (IQR: 88—2207). This was similar to the median time to development of vvGVHD of 452 days in a case series of 19 pediatric post-HSCT patients[10] and longer than the median time of 267 days in a case series of 33 adult female post-HSCT patients.[6] While the median time to vvGVHD in

our study was just over one year after HSCT, it must be noted that Case 1 was diagnosed six years after HSCT.

The incidence of vvGVHD among all female patients who underwent HSCT during the study period was lower than that in the Cizek study (1.2% vs 6.3%), possibly due to under-reporting of symptoms and under-referral in our setting. [10] Symptoms and presentation of vvGVHD were similar in character but less in severity when compared to the Cizek study. While one of our cases was Grade 1 by the Stratton Scale (a three-point scale), one was Grade 2, and two were Grade 3, 89% of patients in the Cizek study were Grade 3 by the Stratton Scale. [6,10] While the clinical presentation of three of our cases were similar to those in adult women, Case 1 had a more classic pediatric vulvovaginitis presentation with development of labial scar tissue, which is more specific for vvGVHD. There are a variety of challenges unique to pediatric post-HSCT populations that could lead to a higher rate of under-diagnoses of vvGVHD when compared to adult women. In addition to the challenges discussed above, no cohesive guidelines currently exist for vvGVHD surveillance in pediatric populations as they do for more common sequelae of HSCT. In a 2015 review on gynecologic care after HSCT, some pediatric screening recommendations are provided, including clinical assessment by a pediatric gynecologist and/or endocrinologist with Tanner staging, inspection of external genitalia and reevaluation every 3-6 months.[23] Cizek and colleagues recommend that female pediatric HSCT patients should receive frequent screening for vvGVHD starting at routine 100 days post-transplant visits, including patients who are asymptomatic.[10]

To our knowledge this is the first study of genital examination patterns for post-HSCT patients at-risk for vvGVHD, furthermore, this is the second to attempt to establish incidence of vvGVHD in a pediatric post-HSCT population. The study was conducted in a pediatric hospital population with good access to PAG specialists and a variety of other sub-specialists involved in the interdisciplinary care of post-HSCT patients. There were several limitations to this study. While we did identify four cases of vvGVHD in the study population, our sample size was

relatively small. It is possible that the true incidence of vvGVHD in this population is much higher than our estimates, especially less symptomatic forms of the disease. While vvGVHD typically occurs in the context of GVHD involving another organ system,[6,8] there are reported cases of isolated vvGVHD.[8] Because we included only patients who had GVHD involving another organ system, we may have missed cases of new-onset vvGVHD occurring in isolation.

Patients who underwent HSCT as children are surviving much longer than in past decades and are therefore developing long-term sequelae that were previously rarely seen. Furthermore, PAG is a relatively new specialty within obstetrics and gynecology and not all pediatric specialties are aware of PAG as a resource for post-HSCT patients. While we did categorize our four vvGVHD cases according to the Stratton and Cizek criteria[6,10] for vvGVHD and vulvar GVHD, respectively (Table 8), clinical information available in the charts was limited and case descriptions may be incomplete. Furthermore, diagnoses of vvGVHD relied on interpretation of documentation from clinical encounters: no patients had photos of vulvovaginal lesions available in their electronic medical record. Similarly, we relied on documentation of genital exams usually performed by non-PAG providers. While it does appear that these providers performed frequent genital examinations, it is possible that the genital exam was part of progress note templates in the electronic medical record, and thus this may be an over-estimate of the number of exams performed. In-depth knowledge of providers on distinguishing normal variants from pathological findings in pediatric vulvovaginal exams may also be limited.

It is likely that both our study and that of Cizek et al.[10] have underestimated the true incidence of vvGVHD in pediatric post-HSCT patients. Thus, larger prospective studies are needed to both determine the true incidence and to elucidate the effectiveness of screening regimens. Many institutions provide post-HSCT “day 100” visits to screen for complications, including GVHD, and these visits should include vulvar exams, even in asymptomatic patients. Furthermore, future research should attempt to clinically differentiate vvGVHD from POI, as signs and symptoms of these conditions can overlap.

Pediatric cancer survivors require interdisciplinary care teams to provide surveillance and management for the multitude of conditions for which they are at increased risk. Given the rarity but severity of vvGVHD, girls who are post-HSCT should have surveillance for vvGVHD by a provider who is trained in identifying and treating vvGVHD, such as gynecologists, pediatricians, oncologists and/or family physicians. Surveillance for vvGVHD should be increased if a patient develops GVHD of the skin and/or a mucosal surface (e.g. oral mucosa). Patients with symptoms consistent with POI must receive a thorough gynecologic exam prior to symptoms being attributed solely to POI. In addition to performing regular exams, providers should frequently inquire about vulvovaginal symptoms. Such screening and surveillance should continue indefinitely, as post-HSCT patients can develop GVHD years after their transplant. Referral to PAG, when accessible, is important at some point in the post-HSCT period to discuss prevention and management of gynecologic sequelae, including vvGVHD, POI, and fertility and sexual health effects. As PAG physicians are few in number and typically located in academic centers, training of and partnership with other practitioners who see these patients is crucial for early detection and treatment of vvGVHD.

**Table Legends**

Table 2. Transplant Characteristics

[a] Includes the following degrees of HLA matching: 10/10, 6/6

[b] Includes the following degrees of HLA matching *and* any transplant from a parent, regardless of degree of HLA match: 3/6, 5/10

[c] includes the following degrees of HLA matching: 4/6, 5/6, 7/10, 9/10

[d] Includes the following degrees of HLA matching: 5/6, 9/10

Table 3. Types of GVHD Prophylaxis

[a] Proportions do not add up to 100%, as many patients received >1 prophylactic medication

Table 4. Sites of GVHD

[a] Includes one that was probable but not confirmed (patient died before case could be confirmed on biopsy)

Table 5. Pediatric and Adolescent Gynecology (PAG) and General Gynecology Referral Characteristics

[a] only includes those that were referred to PAG or General Gyn (n=38)

[b] Included those presenting with other laboratory or imaging abnormalities, well woman exams (no complaints) or those for whom reason for referral was missing

Table 6. Vulvovaginal GVHD Case Descriptions

[a] **Adult vvGVHD grading scale by Stratton [6]:** GRADE 1 (Minimal): Generalized erythema and edema of vulvar structures; patchy erythema of mucosa and glandular structures of vulvar vestibule; erythema around openings of vestibular (Bartholin's & Skene's) glands; vulvar redness, pain on touching the labia, small areas of vulvar denudation (plaques); GRADE 2 (Moderate): Grade I findings plus erosions of mucosal surfaces of the vulva fissures in vulvar folds (e.g., interlabial sulci; fourchette); extensive areas of vulvar denudation with or without leukokeratosis and introital stenosis; Grade I findings plus erosions of mucosal surfaces of the vulva, fissures in vulvar folds (eg, interlabial sulci; fourchette); extensive areas of vulvar denudation with or without leukokeratosis and introital stenosis; GRADE 3 (Severe): Vaginal adhesions or complete vaginal closure; Grade II findings plus agglutination of clitoral hood, introital stenosis, vaginal synechiae, hematocolpos, or complete vaginal closure; Fasciitis or spasticity of levator sling

[b] **Pediatric vulvar GVHD grading scale by Cizek [10]**: GRADE 1: Erythema of vulvar structures, with or without symptoms; GRADE 2: Mild adhesive disease (thin adhesions); presence of scattered skin erosions/fissures; GRADE 3: Moderate adhesive disease (thick/diffuse adhesions, distorting architecture); scattered skin erosions or fissures; GRADE 4: Severe adhesive disease (partial or complete occlusion of urethra and/or vaginal opening); diffuse skin erosions or fissures; loss of architecture of vulvar structures

[c] Case 2 first had a diagnosis of Ewing Sarcoma at age 4, had chemotherapy, later developed Acute Myeloid Leukemia and Myelodysplastic Syndrome at age 7 years

[d] Also had CD34 stem cell top-off three months after PBSCT

**Figure Legends**

Figure 1a: GVHD Timeline for vvGVHD Case 1
[a] Hemophagocytic lymphohistiocytosis syndrome

Figure 1b: GVHD Timeline for vvGVHD Case 2
[a] Acute myeloid leukemia and myelodysplastic syndrome

Figure 1c: GVHD Timeline for vvGVHD Case 3
[a] Acute myeloid leukemia

Figure 1d: GVHD Timeline for vvGVHD Case 4
[a] Acute myeloid leukemia
[b] Had a GVHD/engraftment syndrome phenomenon on the skin the day after transplant; resolved quickly with steroids

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Table 1. Indications for Hematopoietic Stem Cell Transplant

| | N (%) |
|---|---|
| **Indication for HSCT** | |
| Malignant conditions | 55 (64%) |
| Non-malignant conditions | 31 (36%) |
| **Indication for HSCT** | |
| Acute Myeloid Leukemia | 15 (17%) |
| Acute Lymphocytic Leukemia | 30 (35%) |
| Myelodysplastic Syndrome | 3 (3%) |
| Chronic Myelogenous Leukemia | 2 (2%) |
| Non-Hodgkin's Lymphoma | 2 (2%) |
| Hodgkin's Lymphoma | 3 (3%) |
| Severe Aplastic Anemia or other bone marrow failure | 14 (16%) |
| Sickle cell or Thalassemia | 5 (6%) |
| Immune deficiency diseases | 11 (13%) |
| Inherited metabolic disorders | 1 (1%) |

Table 2. Transplant Characteristics

| Characteristic | N (%) |
|---|---|
| **Age at indication diagnosis** (IQR) | 5.1 years (1.6—10.9) |
| Malignant conditions | 7.4 years (3.3—12.5) |
| Non-malignant conditions | 1.1 years (0.3—5.1) |
| **Age at transplant** (IQR) | 7.5 years (3.7—12.7) |
| Malignant conditions | 9.2 years (4.7—14.8) |
| Non-malignant conditions | 4.3 years (1.0—7.4) |
| **Time from indication diagnosis to transplant** (IQR) | 242 days (121—798) |
| Malignant conditions | 259 days (125—798) |
| Non-malignant conditions | 234 days (90—1468) |
| **Type of transplant** | |
| Bone marrow (BMT) | 61 (71%) |
| Peripheral blood stem cell (PBSCT) | 15 (17%) |
| Umbilical Cord blood | 10 (12%) |
| **Source of transplant** | |
| Matched unrelated donor [a] | 25 (29%) |
| Matched related donor (i.e. sibling) [a] | 30 (35%) |
| Haploidentical donor (i.e. parent) [b] | 14 (16%) |
| Mismatched unrelated donor [c] | 14 (16%) |
| Mismatched related donor [d] | 3 (3%) |

Table 3. Types of GVHD Prophylaxis

| | N (%) [a] |
|---|---|
| Tacrolimus | 42 (49%) |
| Cyclosporine | 29 (34%) |
| Methotrexate | 26 (30%) |
| Corticosteroids | 11 (13%) |
| Mycophenolate Mofetil | 11 (13%) |
| Alemtuzumab | 4 (5%) |
| Anti-Thymocyte Globulin | 1 (1%) |
| Other | 5 (6%) |
| No GVHD prophylaxis | 5 (6%) |
| Prophylaxis unknown | 7 (8%) |

Table 4. Sites of GVHD

| **GVHD Site** | Evidence of GVHD at a given site (+/- at other sites), N (%) | Evidence of GVHD at a solitary site, N (%) |
|---|---|---|
| Skin | 75 (87%) | 38 (51%) |
| Lung [a] | 5 (6%) | 1 (20%) |
| Liver | 20 (23%) | 3 (15%) |
| Eyes | 4 (5%) | 0 (0%) |
| Intestine [a] | 22 (26%) | 3 (14%) |
| Oral | 17 (20%) | 3 (18%) |
| Vulvovaginal | 4 (5%) | 0 (0%) |

Table 5. Pediatric and Adolescent Gynecology (PAG) and General Gynecology Referral Characteristics (n=86)

| PAG Characteristic | N (%) |
|---|---|
| Referred to PAG? | 31 (36%) |
| Seen by PAG? | 28 (33%) |
| Referred to General Gynecology? | 7 (8%) |
| Seen by General Gynecology | 3 (3%) |
| Reasons for PAG or General Gynecology referral [a] | |
| Premature ovarian failure | 12 (32%) |
| AUB or other bleeding problem | 11 (29%) |
| Primary amenorrhea or delayed puberty | 3 (8%) |
| Vulvovaginal problem | 5 (13%) |
| Other [b] | 7 (18%) |

Table 6. Vulvovaginal GVHD Case Descriptions

| Case ID | Indication Diagnosis and Transplant Type | Case Description | Adult vvGVHD Grade (1 – 3) [a] | Pediatric Vulvar GVHD Grade (1 – 4) [b] |
|---|---|---|---|---|
| 1 | Griscelli Syndrome & Hemophagocytic Lympho-histiocytosis<br>➔ BMT from matched unrelated donor at age 2 years | - GVHD prophylaxis: cyclosporine, methylprednisolone, tacrolimus<br>- Chronic GVHD of intestine and skin diagnosed 8 months after BMT<br>o GVHD treatment: tacrolimus, etanercept, budesonide, methylprednisolone, topical tacrolimus and triamcinolone<br>- Presented to PAG clinic 5.25 years after BMT (age 8 years) with vulvar itching; cultures grew Gamma-hemolytic Streptococcus species, Gram-negative bacilli and Corynebacterium species; prescribed steroid barrier cream containing hydrocortisone, bacitracin, nystatin and zinc oxide<br>- Vulvar pruritus persisted for one year despite antibiotics and occasional use of barrier cream as needed<br>- Diagnosed with vvGVHD by PAG due to appearance of labial scar tissue, treated with daily application of barrier cream described above, led to symptomatic improvement<br>- No diagnosis of POI during her clinical course; at time of data collection she was 10 years old and had not yet reached menarche | Grade 3 | Grade 2 |
| 2 | Acute Myeloid Leukemia & Myelodysplastic Syndrome [c]<br>➔ PBSCT from haploidentical | - GVHD prophylaxis: none documented<br>- Day 21 after transplant, patient had dysuria, on exam 1-2mm white plaque noted on right labia majora<br>- Day 45, patient complained of “pain in the genital area”, on exam there was a small pearly white papule on clitoris<br>- Day 74, diffuse maculopapular rash with cephalocaudal spread, buccal biopsy showed mild | Grade 1 | Grade 1 |

| | | | | |
|---|---|---|---|---|
| | matched parent at age 7 years | GVHD or oral mucosa<br>- Trial of corticosteroids led to improvement of rash, but when steroids were tapered, rash returned, was more marked, also appeared on vulva; vvGVHD diagnosed by dermatology<br>- Treatment of vvGVHD included topical triamcinolone and tacrolimus, led to clinical improvement<br>- Patient was later diagnosed with POI at age 10 and was started on transdermal estradiol; menarche was at age 12 | | |
| 3 | Acute Myeloid Leukemia<br>➔ BMT from matched unrelated donor, age 17<br>➔ PBSCT from haploidentical donor, age 18 | - GVHD prophylaxis: tacrolimus and unspecified steroids after BMT; tacrolimus, mycophenolic acid, cyclophosphamide after PBSCT<br>- Day 84 after PBSCT, diagnosed with GVHD of intestine<br>- Day 226, admitted to hospital for fever, rash, elevated liver function tests, concern for GVHD. During hospitalization had "vaginal pain", tightness of mons and labia majora, PAG consulted: hypopigmentation in interlabial sulci and tenderness to palpation noted on exam, declined speculum exam. Biopsy of posterior thigh on Day 284 showed skin GVHD<br>o Intestinal and skin GVHD were treated with methylprednisolone, tocilizumab, rituximab, etanercept, basiliximab, and topical clobetasol<br>o Treated for concomitant vvGVHD with topical clobetasol and mometasone with clinical improvement; no further vulvovaginal complaints<br>- Had laboratory evidence of decreased ovarian reserve, no formal diagnosis of POI (was on leuprolide at time of testing); menarche had been at age 13 (prior to AML diagnosis) | Grade 2 | Grade 2 |
| 4 | Acute Myeloid Leukemia<br>➔ BMT from matched unrelated donor, age 17 | - Seen by PAG after AML diagnosis but before BMT for menstrual suppression (menarche was at 13), started on leuprolide which she continued until 6 months after transplant<br>- GVHD prophylaxis: cyclosporine and unspecified steroids<br>- Day 31 after transplant, diagnosed with GVHD of intestine<br>o Treated with methylprednisolone, prednisone, etanercept, ruxolitiab, oral budesonide<br>- Day 134, diagnosed with GVHD of skin and oral mucosa<br>o Skin GVDH treated with imatinib, topical tacrolimus, unspecified steroids (IV, topical) | Grade 3 | Grade 3 |

| | | | | |
|---|---|---|---|---|
| | | o Oral: topical barrier creams and oral dexamethasone<br>- Day 274, saw PAG for vulvar pain/sensitivity/dryness, on exam vulva appeared hypoestrogenic, otherwise normal, internal exam declined. Labs consistent with POI, started on oral contraceptive pills for hormone replacement<br>- Day 371, diagnosed with GVHD of lung, specifically bronchiolitis obliterans<br>o Treated with: methylprednisolone, inhaled corticosteroids and bronchodilators, azithromycin prophylaxis<br>- Day 475, saw PAG again for persistent vulvar pain; mild labial adhesions noted on exam, was started on topical estradiol<br>- Day 512, follow up with PAG; worsening labial adhesions (approximately 60% agglutinated) despite topical estradiol use, adhesions attributed to vvGVHD<br>- Day 1310, underwent exam under anesthesia and repair of labial agglutinations with PAG; exam showed normal appearing majora, resorption of labia minora bilaterally, thick agglutinated labial adhesions 85% posteriorly<br>- In post-operative follow up, continued to use topical estrogen and zinc oxide barrier creams | | |