Histologic and clinical cross-sectional study of chronic hair loss in patients with cutaneous chronic graft-versus-host disease

Dustin H. Marks, BS,[a] Jason S. Naftulin, MS,[a] Lauren R. Penzi, MD,[a] Athena Manatis-Lornell, BA,[a] Mariko R. Yasuda, MD,[a,b] Clifford M. Chapman, MS, HTL(ASCP), QIHC,[c] Sowmya R. Rao, PhD,[d] Arturo Saavedra, MD,[e] and Maryanne M. Senna, MD[a,b]
Boston and Woburn, Massachusetts, and Charlottesville, Virginia

Background: While scalp alopecia represents a distinctive feature of chronic graft-versus-host disease (cGVHD), little is known about the clinical and histologic presentation of hair loss.

Objectives: We sought to classify the clinical presentations and histologic findings of chronic hair loss in patients with cutaneous cGVHD.

Methods: A prospective cohort of 17 adult hematopoietic cell transplantation (HCT) recipients with cutaneous cGVHD was enrolled. Dermatologic examinations were performed, and punch biopsy specimens of the scalp were obtained. Biopsy specimens were analyzed with hematoxylin–eosin and immunohistochemical stains in all cases and fluorescence in situ hybridization analyses in specific cases.

Results: Clinically, 4 patterns of hair loss were described—patchy nonscarring (41.2%), diffuse nonscarring (11.8%), diffuse sclerotic (11.8%), and patchy sclerotic (5.9%). The location of the inflammatory infiltrate on hematoxylin–eosin-stained specimens correlated with the hair loss pattern patients had clinically, with cell populations around the bulb and bulge in nonscarring and sclerotic cases, respectively. Fluorescence in situ hybridization studies in female cGVHD patients with male donors demonstrated green Y chromosomes limited to the area of the hair follicle affected by inflammatory cells.

Conclusion: This study describes the various clinical and histologic subtypes of long-standing alopecia in adult cGVHD patients and suggests that this alopecia may be a direct manifestation of cGVHD of the hair follicle. (J Am Acad Dermatol 2019;81:1134-41.)

Key words: alopecia; chronic graft-versus-host disease; cutaneous graft-versus-host disease; fluorescence in situ hybridization; hair loss; noncicatricial alopecia.

INTRODUCTION

Chronic graft-versus-host disease (cGVHD) remains a prevalent and pernicious complication impacting long-term survivors of allogenic hematopoietic cell transplantation (HCT).[1] Occurring in 40% to 70% of allograft recipients, cGVHD represents the leading cause of mortality in patients surviving >2 years after transplantation.[2-4] Individuals with cGVHD also experience significant physical, emotional, and functional limitations associated with their disease state that hold critical consequences for their overall quality of life.[5]

From the Department of Dermatology,[a] Massachusetts General Hospital, Harvard Medical School,[b] and MGH Biostatistics Center,[d] Boston; Med-Sci Consultants,[c] Woburn; and the Department of Dermatology, University of Virginia, Charlottesville.[e]

Funding sources: None.
Conflicts of interest: None disclosed.
Accepted for publication March 8, 2019.

Reprints not available from the authors.
Correspondence to: Maryanne M. Senna, MD, Department of Dermatology, Massachusetts General Hospital, 50 Staniford St, Ste 200, Boston, MA 02114. E-mail: msenna@partners.org.

Published online August 24, 2019.
0190-9622/$36.00

https://doi.org/10.1016/j.jaad.2019.03.031

Cutaneous cGVHD, furthermore, may manifest in a variety of forms—from a lichenoid eruption to deep sclerotic involvement with ulceration.[1] Both clinical and histopathologic analyses have long served as standard tools for the diagnosis of cutaneous cGVHD.[6] In addition, fluorescence in situ hybridization (FISH) using sex chromosome probes represents a rapid and reliable test to confirm the diagnosis of cutaneous cGVHD in sex-mismatched transplants.[7,8]

Although the appreciation of clinical manifestations and diagnostic methods of cutaneous cGVHD continues to expand, there remains a paucity of literature detailing associated adnexal involvement. Hair loss after allogenic transplant, moreover, is not infrequent and may result from several causes, including chemotherapy-induced alopecia, medication side effects, infection, metabolic or endocrine dysfunction, telogen and anagen effluvium, alopecia areata, or GVHD.[9]

In addition, new scarring or nonscarring alopecia, hair thinning, hair brittleness, loss of body hair, and premature graying represent distinctive or associated features of cGVHD but are insufficient alone to establish a diagnosis.[10] While the pathogenesis of these hair changes is ill-defined, it is hypothesized that the perifollicular hair unit may function as an early target in GVHD, resulting in hair disturbances. In accordance, limited case reports have described scarring and nonscarring scalp alopecia as distinctive clinical manifestations of cGVHD. In addition, there have been 3 biopsy-supported cases of cGVHD presenting as either scarring or nonscarring alopecia.[9,11] However, there are currently no reports in the literature detailing the variety of clinical presentations of cGVHD alopecia and the corresponding histopathologic findings. The use of FISH analysis in scalp biopsy speciemens of patients with cGVHD and long-standing alopecia has also not been previously reported.

In the current study, the histologic and clinical characteristics of patients with cGVHD and long-standing hair loss were recorded and analyzed to support the manifestation of cGVHD as a distinctive form of alopecia.

CAPSULE SUMMARY

- Long-standing scalp alopecia is seen in adult patients with chronic graft-versus-host disease (cGVHD), but the clinical presentations and histologic findings of this type of hair loss are unknown.
- This study describes the clinical presentations and histologic findings of alopecia in patients with cGVHD.
- Fluorescence in situ hybridization analyses in female patients with cGVHD who had male stem cell donors suggest that their alopecia may be a direct manifestation of cGVHD of the hair follicle.

METHODS

This is a single-center, cross-sectional observational prospective cohort study of adult HCT recipients with a diagnosis of cutaneous cGVHD at Massachusetts General Hospital (MGH) in Boston, Massachusetts. Patients were seen over a 3-month timeframe from June 2017 to August 2017 and followed by a dermatologist specializing in cutaneous cGVHD.

Patients 18 to 80 years of age who were recipients of allogeneic HCTs and who had received a subsequent diagnosis of cutaneous cGVHD, as established by the diagnostic criteria in the National Institutes of Health Consensus Development, were included in the study. Subjects were excluded from the study if they had >2 HCTs, HCT(s) performed outside of MGH, a personal history of hypersensitivity to local anesthetics, or if they were pregnant, nursing, or planning to become pregnant. Institutional review board approval through the Partners Human Research Committee at MGH was obtained, as was written informed consent from the patient in all cases.

During visits to the GVHD dermatology clinic at MGH, eligible patients were recruited and enrolled in the study. Participants were clinically evaluated by 2 dermatologists (AS, MMS) who performed a complete dermatologic evaluation of the skin, mucous membranes, hair, and nails. Detailed attention was given to alopecia distribution and severity, hair and scalp characteristics, and evaluation of other hair-bearing sites. All clinical findings of alopecia deemed related to cGVHD were photographed and documented in the patient chart.

If enrolled patients were found to have cGVHD-related alopecia on clinical examination, two 4-mm punch biopsy specimens of the scalp were obtained if the patient was considered stable enough to undergo the procedure in the opinion of the investigators. Scalp biopsy specimens were analyzed using hematoxylin—eosin (H&E) and immunohistochemical (IHC) stains in all cases. IHC analysis included CD3 (T cell), CD4, CD5 (activated or regulatory T cell), CD8, CD20 (B cell), CD30 (activated T cell), CD123 (plasmacytoid dendritic cells),

Table I. Demographics and clinical data

Sex	Age at time of study visit, y	Primary disease	Age at time of HCST, y	Induction regimen	Donor type
M	42	Refractory anemia	41	Fludarabine/melphalan	Sex concordant, HLA match
M	49	Hodgkin lymphoma	43	Busulfan/fludarabine	Sex concordant, HLA match
M	25	AML	25	Busulfan/cyclophosphamide	Sex concordant, HLA match
F	59	AML	57	Busulfan/fludarabine	Sex concordant, HLA match
M	73	AML	70	RIC busulfan/fludarabine	Sex discordant, HLA match
M	70	MDS	66	Busulfan/fludarabine	Sex concordant, HLA match
M	46	AML	40	Larson regimen	Sex concordant, HLA match
F	64	ALL	59	Busulfan/clofarabine	Sex discordant, HLA match
M	50	Diffuse large B cell lymphoma	47	TBI/cyclophosphamide	Sex concordant, HLA match
F	63	AML	60	Fludarabine/melphalan/RGI	Sex discordant, HLA match
F	61	AML	57	Fludarabine/melphalan	Sex concordant, HLA match
F	62	AML	61	RIC busulfan/fludarabine	Sex discordant, HLA match
M	62	CLL	59	Fludarabine/melphalan	Sex concordant, HLA match
M	47	AML	46	Busulfan/fludarabine	Sex concordant, HLA match
F	48	AML	46	Cytarabine/daunorubicin	Sex discordant, HLA match
F	67	AML	61	RIC busulfan/fludarabine	Sex concordant, HLA match
F	66	Myelofibrosis	63	RIC fludarabine/melphalan	Sex discordant, HLA match

ALL, Acute lymphocytic leukemia; *AML*, acute myeloid leukemia; *F*, female; *CLL*, chronic lymphocytic leukemia; *HCST*, hematopoietic stem cell transplant; *HLA*, human leukocyte antigen; *M*, male; *MDS*, myelodysplastic syndrome; *RIC*, reduced intensity conditioning; *TBI*, total body irradiation.

Table II. Prospective study hair and nail chronic graft-versus-host disease characteristics

Hair characteristic type	n (%)	Concomitant AGA or FPHL, n (%)
Normal hair	5 (29.4)	2 (40)
Patchy nonscarring	7 (41.2)	2 (28.6)
Diffuse nonscarring	2 (11.8)	0
Patchy sclerotic	1 (5.9)	0
Diffuse sclerotic	2 (11.8)	1 (50)

AGA, Androgenetic alopecia; *FPHL*, female pattern hair loss.

CK15 (bulge stem cells), and FOXP3 (regulatory T cell).

In scalp biopsy specimens from female HCT patients with male donors, FISH analysis was performed for XY chromosomes using Vysis DNA centromere probes (Abbott Molecular Inc, Des Plaines, IL) for X (red, recipient) and Y (green, donor) chromosomes. This analysis was used to identify chimerism and investigate the presence and distribution of donor chromosomes in the scalp skin and hair follicle in sex-mismatched transplant patients.

The medical records of participants were reviewed and additional data were collected. The following clinical data were included: age, sex, primary indication for HCT, source of HCT, frequency of HCT, conditioning regimen, human leukocyte antigen match, sex of donor, history of acute GVHD, and past and current corticosteroid and immunosuppressive medications.

RESULTS

Seventeen adult HCT recipients with cutaneous cGVHD were enrolled in this prospective, cross-sectional study (Table I), including 8 women and 9 men with a combined mean age of 56.1 years (standard deviation, 12.3 years). Acute myeloid leukemia (58.8%) was the most frequent indication for HCT. The most prevalent conditioning regimens encompassed busulfan/fludarabine (41.2%) and fludarabine/melphalan (29.4%). Most donors (64.7%) were sex-concordant and human leukocyte antigen–matched. At the time of study participation and the time of obtaining the biopsy specimen (if obtained), the majority of patients (76.5%) reported current treatment with systemic corticosteroids or immunosuppressive agents, including prednisone (52.9%), tacrolimus (52.9%), mycophenolate mofetil (17.7%), sirolimus (11.8%), and methotrexate (5.9%).

Clinical characteristics of hair cGVHD

The clinical characteristics of long-standing hair loss in the reported 17 patients with cutaneous cGVHD are summarized in Table II. The average duration of persistent scalp hair loss was 32 months (range, 6-87 months). Most patients displayed patchy nonscarring alopecia (41.2%) or had normal hair

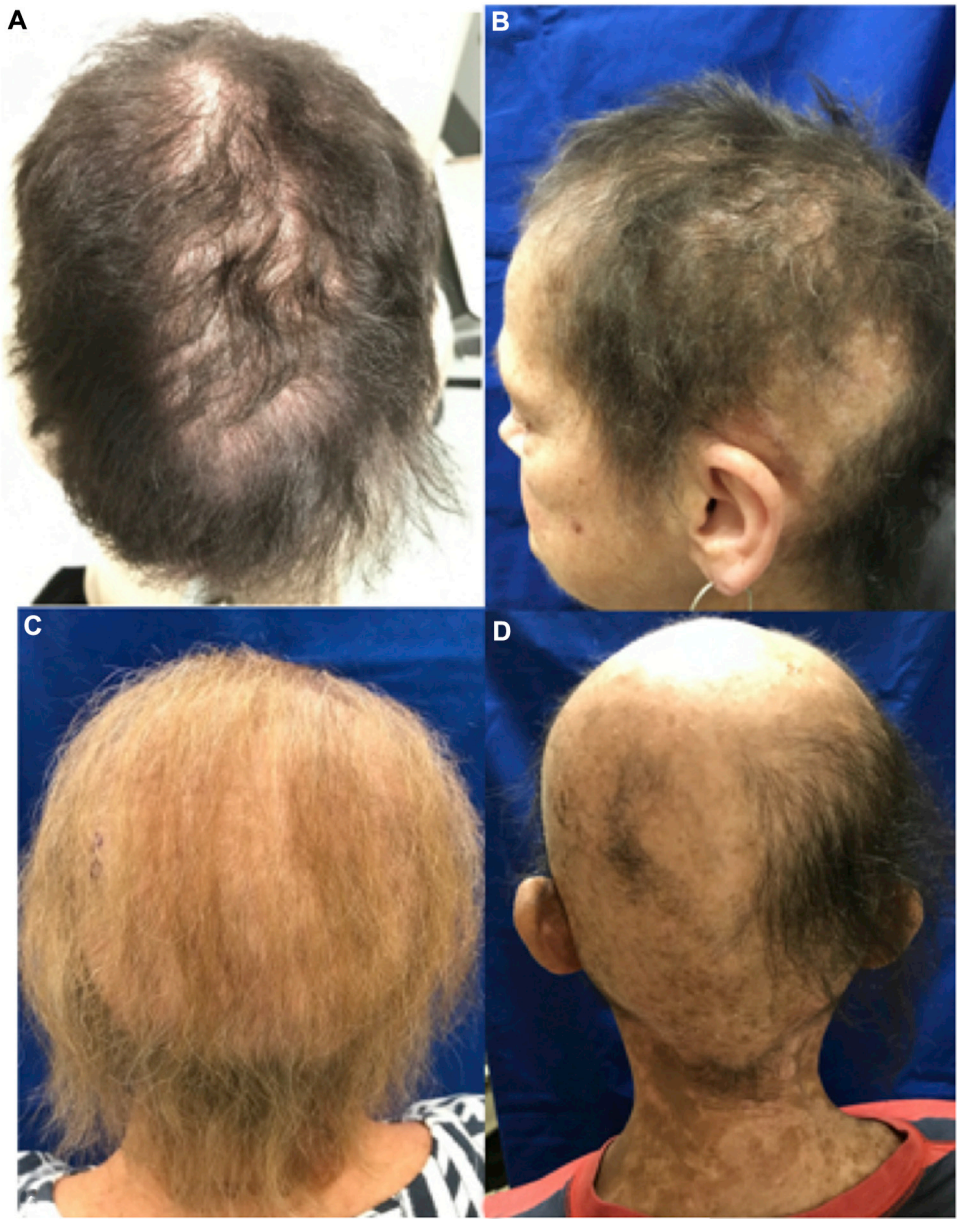

Fig 1. Clinical hair findings in patients with chronic graft-versus-host disease. **A**, Patchy nonscarring alopecia. **B**, Patchy sclerotic alopecia. **C**, Diffuse nonscarring alopecia. **D**, Diffuse sclerotic alopecia.

(29.4%). Other patterns of hair loss included diffuse nonscarring (11.8%), diffuse sclerotic (11.8%), and patchy sclerotic (5.9%) alopecia. Examples of these 4 clinical hair loss patterns are shown in Fig 1. Typical manifestations of concomitant androgenetic alopecia or female pattern hair loss were noted in 25% of patients with another distinct pattern of alopecia as described above. There was no significant relationship between use of corticosteroids or other immunosuppressive agents with the presence or type of hair loss.

Histologic findings

Histologic analysis of scalp samples using H&E staining in all cases revealed intact hair follicles with increased catagen and telogen hairs. The location of the inflammatory infiltrate correlated with the type of hair loss pattern patients were noted to have clinically. In patients with nonscarring forms of hair loss, inflammatory pathology was displayed at the level of the hair bulb matrix (Fig 2). This location of inflammation contrasted with patients with clinically sclerotic hair loss who were found to have

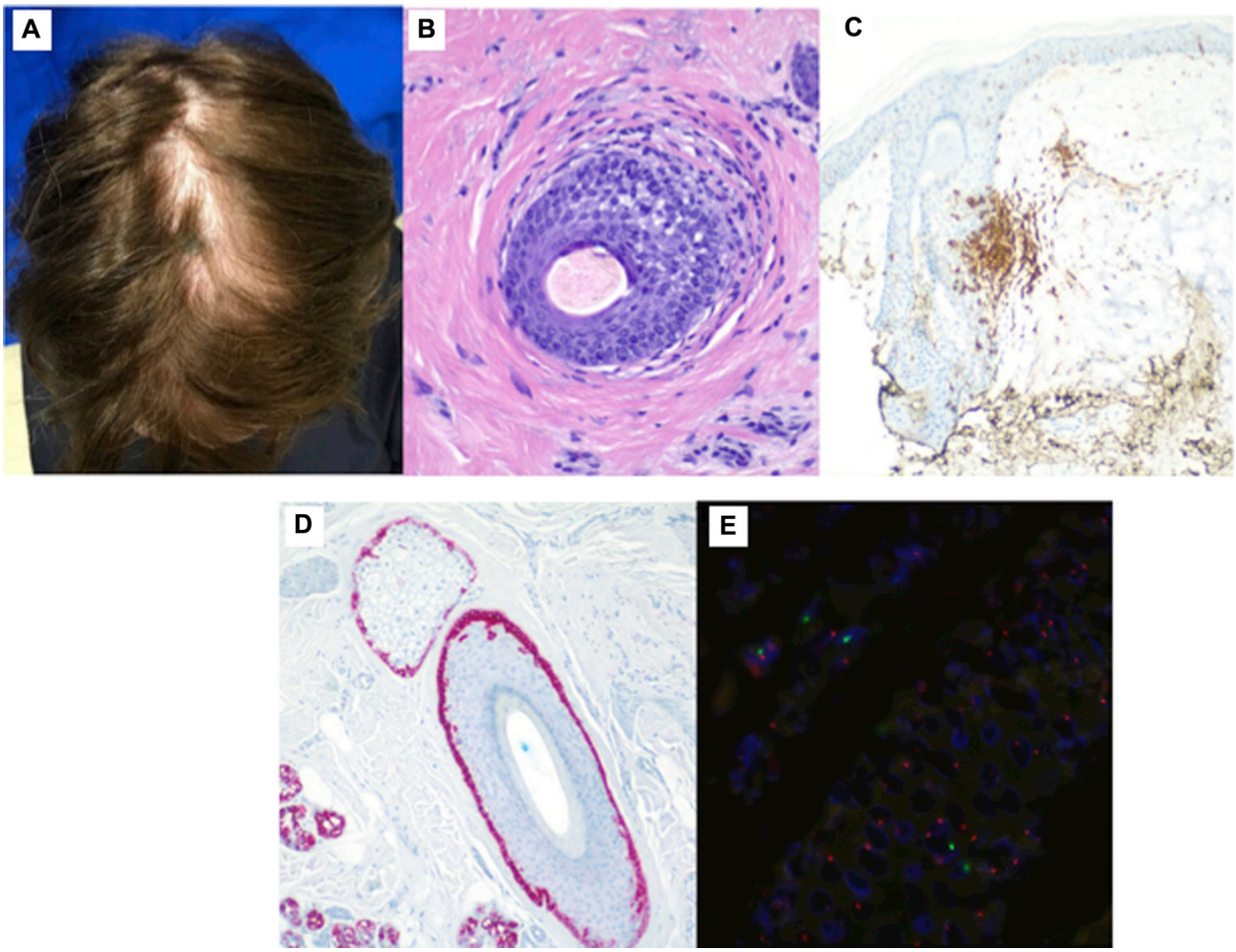

Fig 2. Clinical and histologic findings of "alopecia areata–like" chronic graft-versus-host disease alopecia. **A**, Nonscarring alopecia on the top of the scalp. **B**, Hair follicle with peribulbar lymphocytes on hematoxylin–eosin staining. **C**, Immunohistochemistry showing a predominantly CD4 T cell infiltrate. **D**, Immunohistochemistry showing strong $CK15^+$ staining. **E**, Fluorescence in situ hybridization demonstrating green (Y chromosomes) in peribulbar location.

inflammatory activity at the level of the insertion of the arrector pili muscle (ie, the bulge region) in addition to perifollicular concentric fibrosis (Fig 3). All specimens—including both sclerotic and nonscarring alopecia patterns—contained identical immunohistochemical staining with $CD4^+$ $CD25^+$ $FOXP3^-$ T cell infiltrate (CD4:CD8 ratio, 10:1). Similarly, all specimens exhibited diffuse $CK15^+$ expression. CD123 staining was negative. There was no relationship between the use of corticosteroids or other immunosuppressive agents with the histologic findings.

In female cGVHD patients who had male HCT donors, FISH studies demonstrated green Y chromosomes within the epidermis of the skin, consistent with their known diagnosis of cutaneous cGVHD. Y chromosomes were also identified at the precise hair follicle location of the inflammatory infiltrate, surrounding the hair bulb in patients with nonscarring alopecia and at the hair follicle bulge in patients with sclerotic alopecia, respectively. Y chromosomes were not observed as present in any other part of the hair follicular unit.

DISCUSSION

While specific pathology of the skin (ie, poikiloderma, deep sclerosis) serves as diagnostic criteria for cGVHD, new-onset sclerotic or nonscarring scalp alopecia remains a distinctive feature that is alone insufficient to establish a diagnosis. Other hair characteristics also seen in cGVHD include thinning scalp hair—typically patchy, coarse, or dull—premature graying and, most recently reported, acquired pili torti.[10,12] Despite the prevalence and

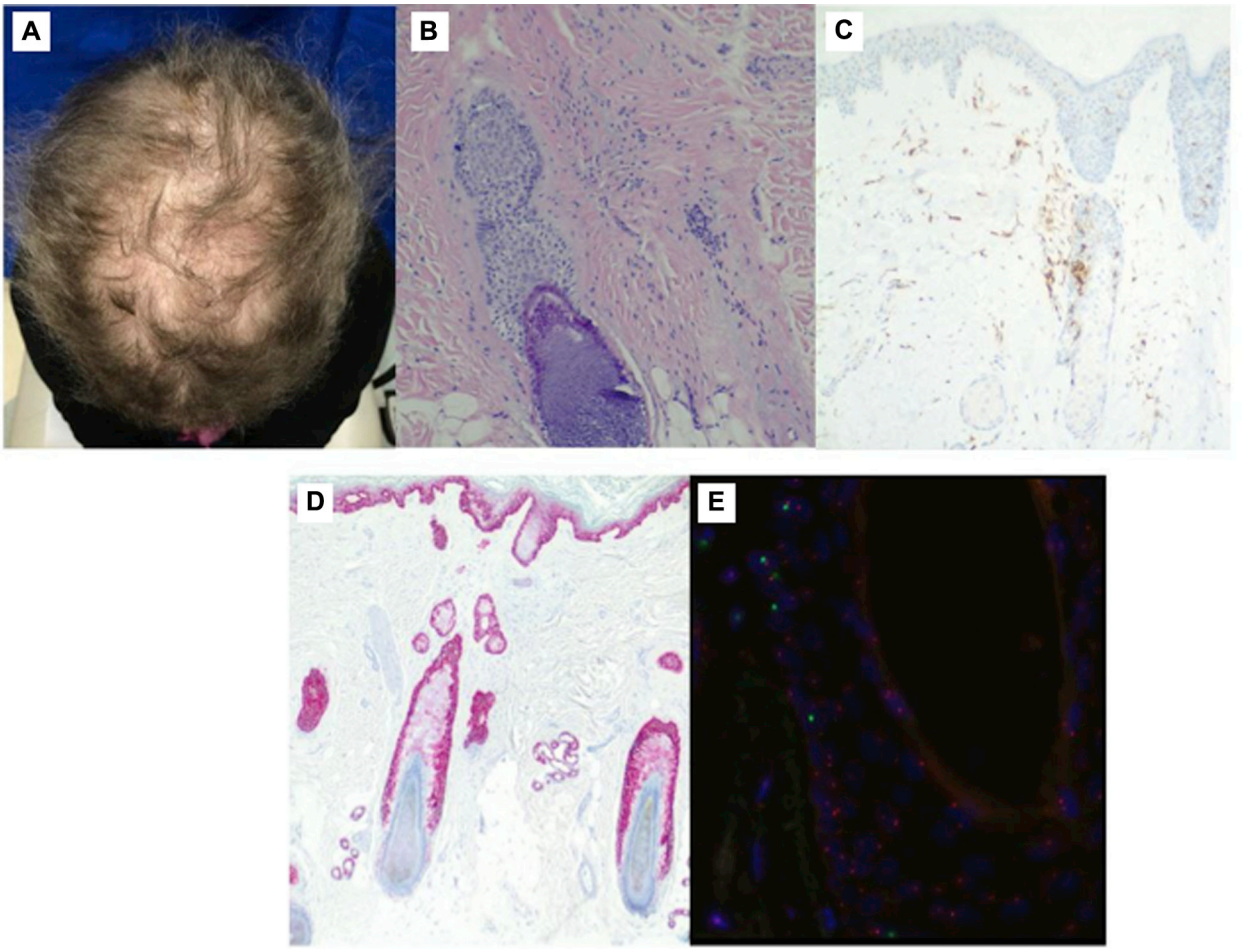

Fig 3. Clinical and histologic findings of "lichen planopilaris–like" chronic graft-versus-host disease alopecia. **A**, Diffuse scarring alopecia on the top of the scalp. **B**, Hair follicle with columnar fibrosis and lymphocytes near isthmus on hematoxylin–eosin staining. **C**, Immunohistochemistry showing a predominantly CD4 T cell infiltrate. **D**, Immunohistochemistry showing strong $CK15^{+}$ staining. **E**, Fluorescence in situ hybridization demonstrating green (Y chromosomes) in a location near the hair follicle bulge.

potential impact of hair involvement, there is no published literature to our knowledge that systematically describes both the clinical presentations and the histopathology of long-standing scalp hair loss in patients with cutaneous cGVHD.[9,11]

In this cohort, 88.2% of patients with cGVHD displayed ≥1 pattern(s) of chronic hair loss. While the minority (11.8%) exclusively had hair loss patterns consistent with androgenetic alopecia or female pattern hair loss, 70.6% exhibited an alternative pattern of hair loss. This high frequency may overestimate the prevalence of scalp alopecia in the general cGVHD population, because patients with hair loss were likely more inclined to participate in the current study. Recognizing that the overall prevalence of long-standing alopecia in patients with cGVHD is likely lower, patients in this study most commonly displayed nonscarring areas of hair loss with patchy or diffuse involvement similar to alopecia areata (AA).[13] Several case reports and a retrospective study have previously described the onset of AA after allogenic HCT, commonly in the setting of cGVHD, and further confirmed a statistical significance with the development of AA and female donors and in particular, female donors to male recipients.[9,14-16] Most of the reported patients, however, displayed hair loss most consistent with diffuse AA as opposed to patchy alopecia that was more frequently observed in the current study.

In contrast to an AA-like presentation, the dermoscopic examination of patients with sclerotic alopecia revealed perifollicular scaling or follicular dropout in 25% of patients in this cohort. Similar presentations in individuals with cGVHD have been

previously reported in limited case reports and series that described clinical characteristics analogous to lichen planopilaris (LPP) and fibrosing alopecia in a pattern distribution.[11,17]

Paralleling the 2 distinct clinical patterns of nonscarring and sclerotic alopecia, histopathologic evaluation of scalp biopsy specimens revealed corresponding regions of inflammatory involvement, respectively. In patchy and diffuse nonscarring presentations, H&E staining demonstrated inflammatory activity surrounding the hair bulb, as in AA.[18] In contrast, the inflammatory cells in sclerotic presentations centered around the hair bulge, as seen in LPP.[19]

Despite distinct differences in the location of the inflammatory infiltrate on H&E, immunohistochemical staining revealed similarities amongst all specimens, suggesting a potentially shared pathogenesis. Interestingly, the ratio of CD4:CD8 cells remained 10:1 across the bulge or bulb in all biopsy specimens. This increase in $CD4^+$ cells is most consistent with, albeit considerably higher than, the CD4:CD8 ratio of 4:1 seen in the involved peribulbar area of AA.[20] In contrast, $CD8^+$ cells dominate in the infundibulum and bulge epithelium of patients with LPP, with a CD4:CD8 ratio of 2.5:1 in areas of inflammation.[19]

Furthermore, the uniform $CD4^+$ $CD25^+$ T cell infiltrate did not express high levels of FoxP3, signifying the absence of regulatory T cells. Previous reports have indicated increased, normal, and decreased numbers of regulatory T cells at the onset of cGVHD. A prospective study of 107 adult patients found that homeostatic imbalance of T cells—namely decreased thymic production of regulatory T cells and the ultimate persistence of effector T cells—was associated with the development of cGVHD.[21]

In addition, IHC staining revealed that all specimens—including those with inflammatory activity concentrated at the hair bulb—were negative for CD123, a marker of interferon-secreting plasmacytoid dendritic cells. Plasmacytoid dendritic cells are abundant in the peribulbar infiltrate in AA[22] and are increased in the perifollicular mesenchyme surrounding the hair infundibulum in LPP.[19,23] All scalp biopsy specimens, furthermore, displayed a robust expression of CK15. Although lacking specificity, CK15 remains the best marker for bulge stem cells and is accordingly lost in many scarring alopecias but preserved in nonscarring forms.[24,25] All specimens in this study showed strong CK15 expression, and therefore it is suggested that the hair follicle bulge stem cells are maintained in cGVHD patients with both patterns of alopecia despite the perifollicular fibrosis observed in patients with sclerotic alopecia. Scalp biopsy specimens were only obtained from patients without extensive involvement and pain who were able to tolerate the procedure, and therefore the findings of $CK15^+$ may not hold true for the most severe forms of alopecia in patients with cGVHD.

Taken together, our findings suggest that the IHC signature in these patients could represent a unique alopecia entity with striking similarities to AA and LPP with respect to the location of the inflammatory infiltrate, but with differing cell populations.

Irrespective of the clinical or histopathologic assessment, FISH studies in female cGVHD patients with sex-mismatched donors unequivocally demonstrated green Y chromosomes directly in the inflammatory infiltrate—whether bulb or bulge—on examination of the scalp biopsy specimen. Y chromosomes were not seen at any other location of the hair follicle or dermis. Of chief importance, this anatomic distribution of Y chromosomes at the respective location of inflammation in the hair follicle bulb or bulge supports the presence of GVHD of the hair follicular unit, which has not been previously identified through use of FISH analysis. Historical evidence supported alopecia as a direct manifestation of cGVHD in the context of patients who presented with newfound clinical and histologic evidence of alopecia with cGVHD in other organs.[9,11,17,26] The use of FISH analysis now further substantiates the existence of cGVHD scalp alopecia. Although we cannot definitively prove that scalp alopecia is a direct manifestation of cGVHD in this study, the anatomic distribution of Y chromosomes at the level of the hair bulge or bulb in female patients with sex-mismatched donors and the paucity of Y chromosomes elsewhere strongly supports a unique form of alopecia caused by cGVHD.

These findings suggest that if cGVHD of the hair follicle represents a distinct type of alopecia, revision to the current diagnostic National Institutes of Health consensus framework should be considered to include new sclerotic or nonscarring alopecia as a diagnostic criterion for cGVHD. Although not all hair loss represents cGVHD, alopecia may be an early, subtle, or singular manifestation of the disease, and if misdiagnosed, this could delay appropriate treatment and rehabilitation.[1] Regardless of the diagnostic criteria, it is recommended that all practitioners routinely examine and record adnexal findings for patients with diagnosed or suspected cGVHD, as a recent case-controlled study found that long-standing alopecia and nail dystrophy in adults was associated with more severe overall cGVHD and increased rates of ocular and oral cGVHD.[27] Patients with long-lasting alopecia may benefit from more frequent monitoring and preemptive treatment strategies for the disease in its entirety.[27]

This study was limited to longstanding hair loss in patients with cGVHD with concomitant cutaneous involvement. Larger prospective trials are indicated to further evaluate hair involvement in patients with cGVHD without other skin findings and patients at risk to develop cGVHD with the theoretical potential to identify newfound alopecia as an early indication of cGVHD. Additional research is imperative to 1) explain the inefficacy of systemic immunosuppressants in the treatment of cGVHD alopecia, 2) identify the target antigen(s) in the hair bulb and bulge regions, and 3) determine the susceptibility of patients to develop long-standing alopecia as a direct manifestation of cGVHD.

In conclusion, while previous case series and reports have documented the presence of alopecia in patients with cGVHD, the current study is the first to describe the various presentations of long-standing hair loss in cGVHD and provide the corresponding histologic classification with both H&E and IHC staining. Our findings support that this long-standing alopecia may be a direct manifestation of cGVHD through use of FISH analysis to identify chimerism in sex-mismatched transplants that has not been previously performed. Despite distinct clinical and histopathologic presentations, cGVHD of the hair follicle is potentially reversible given that all scalp biopsy specimens—including those that are sclerotic in nature—exhibited diffuse CK15 staining. Additional research is needed to determine safe treatment interventions for this form of hair loss.

REFERENCES

1. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2003;9:215-233.
2. Ochs LA, Miller WJ, Filipovich AH, et al. Predictive factors for chronic graft-versus-host disease after histocompatible sibling donor bone marrow transplantation. *Bone Marrow Transplant*. 1994;13:455-460.
3. Lee SJ, Klein JP, Barrett AJ, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. *Blood*. 2002;100:406-414.
4. Socié G, Stone JV, Wingard JR, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. *N Engl J Med*. 1999;341:14-21.
5. El-Jawahri A, Pidala J, Khera N, et al. Impact of psychological distress on quality of life, functional status, and survival in patients with chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2018;24:2285-2292.
6. Favre A, Cerri A, Bacigalupo A, Lanino E, Berti E, Grossi CE. Immunohistochemical study of skin lesions in acute and chronic graft versus host disease following bone marrow transplantation. *Am J Surg Pathol*. 1997;21:23-24.
7. Kanehira K, Riegert-Johnson DL, Chen D, Gibson LE, Grinnell SD, Velgaleti GV. FISH diagnosis of acute graft-versus-host disease following living-related liver transplant. *J Mol Diagn*. 2009;11:355-358.
8. Nguyen J, Tetzlaff MT, Zhang PJ, Xu X, Hexner E, Rosenbach M. Fluorescent in situ hybridization of a skin biopsy: an adjunctive tool to support a diagnosis of graft-versus-host disease. *J Am Acad Dermatol*. 2011;64:e113-e114.
9. Silveira L, Natalia Fraia e Souza M, Teixeira S, et al. Graft-versus-host disease with acute hair loss: a distinctive manifestation of chronic graft-versus-host disease. *J Am Acad Dermatol*. 2016;74:AB135.
10. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21:389-401.e1.
11. Basilio FMA, Brenner FM, Werner B, Rastelli GJC. Clinical and histological study of permanent alopecia after bone marrow transplantation. *An Bras Dermatol*. 2015;90:814-821.
12. Penzi LR, Saavedra A, Senna MM. Long-standing pili torti in 2 patients with chronic graft-vs-host disease. *JAAD Case Rep*. 2017;4:44-46.
13. Finner AM. Alopecia areata: clinical presentation, diagnosis, and unusual cases. *Dermatol Ther*. 2011;24:348-354.
14. Sanli H, Kusak F, Arat M, Ekmekci P, Ilhan O. Simultaneous onset of chronic graft versus host disease and alopecia areata following allogeneic haematopoietic cell transplantation. *Acta Derm Venereol*. 2004;84:86-87.
15. Kamińska ECN, Larson RA, Petronic-Rosic V. Amelanocytic anhidrotic alopecia areata-like phenotype after allogeneic hematopoietic cell transplant. *Arch Dermatol*. 2012;148:931-934.
16. Zuo RC, Naik HB, Steinberg SM, et al. Risk factors and characterization of vitiligo and alopecia areata in patients with chronic graft-vs-host disease. *JAMA Dermatol*. 2015;151:23-32.
17. Rezende HD, Reis Gavazzoni Dias MF, Trüeb RM. Graft versus host disease presenting as fibrosing alopecia in a pattern distribution: a model for pathophysiological understanding of cicatricial pattern hair loss. *Int J Trichology*. 2018;10:80-83.
18. Pierard GE, De la Brassinne M. Cellular activity in the dermis surrounding the hair bulb in alopecia areata. *J Cutan Pathol*. 1975;2:240-245.
19. Harries MJ, Meyer K, Chaudhry I, et al. Lichen planopilaris is characterized by immune privilege collapse of the hair follicle's epithelial stem cell niche. *J Pathol*. 2013;231:236-247.
20. Happle R, Klein HM, Macher E. Topical immunotherapy changes the composition of the peribulbar infiltrate in alopecia areata. *Arch Dermatol Res*. 1986;278:214-218.
21. Alho AC, Kim HT, Chammas MJ, et al. Unbalanced recovery of regulatory and effector T cells after allogeneic stem cell transplantation contributes to chronic GVHD. *Blood*. 2016;127:646-657.
22. Abou Rahal J, Kurban M, Kibbi A-G, Abbas O. Plasmacytoid dendritic cells in alopecia areata: missing link? *J Eur Acad Dermatol Venereol*. 2016;30:119-123.
23. Sleiman R, Kurban M, Abbas O. Evaluation of the diagnostic value of plasmacytoid dendritic cells in differentiating the lymphocytic cicatricial alopecias. *Dermatology*. 2015;231:158-163.
24. Mahalingam M. Lack of specificity of cytokeratin-15 loss in scarring alopecias. *J Am Acad Dermatol*. 2017;76:e135-e136.
25. Hoang MP, Keady M, Mahalingam M. Stem cell markers (cytokeratin 15, CD34 and nestin) in primary scarring and nonscarring alopecia. *Br J Dermatol*. 2009;160:609-615.
26. Cooke KR, Luznik L, Sarantopoulos S, et al. The biology of chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2017;23:211-234.
27. Naftulin JS, Penzi LR, Manatis-Lornell A, et al. Longstanding alopecia and nail dystrophy are associated with more severe overall chronic graft-versus-host disease in adults. *Bone Marrow Transplant*. 2019;54:469-472.