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Gastrointestinal Graft-versus-Host Disease in Recipients of Autologous Hematopoietic Stem Cells: Incidence, Risk Factors, and Outcome

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ABSTRACT

Graft-versus-host disease (GVHD) is seen in skin, intestinal mucosa, and liver after autologous stem cell transplantation. We reviewed 681 consecutive patients to estimate the probability of gastrointestinal (GI) GVHD, response to treatment, risk factors for development, and effect on survival. GI GVHD was defined by persistent symptoms, mucosal abnormalities at endoscopy, and histology showing apoptotic crypt cells with or without lymphoid infiltrates. The proportion of patients with GI GVHD was 90/681 (13%). Nausea and vomiting occurred in 90% and diarrhea in 40%. The mean time to developing symptoms was day +15, that to histologically proven diagnosis was day +42, and that to starting prednisone treatment was day +45 after stem cell infusion. Treatment with a short course of prednisone effected durable responses in 79% of patients, and an additional 18% responded to a second course of prednisone. A multivariable logistic regression model demonstrated that the combined factor of a diagnosis of breast cancer or hematologic malignancy and female sex was statistically significantly associated with the probability of GI GVHD ($P = .003$). Survival in patients with GI GVHD was not statistically different than that in those without GVHD. We conclude that women with breast cancer or hematologic malignancy are more likely to develop GI GVHD after autologous transplantation, and that treatment with prednisone was effective.

KEY WORDS

Hematopoietic cell transplantation • Autologous transplantation • Graft-versus-host disease • Lymphocytic gastritis • Microchimerism • Corticosteroid treatment

INTRODUCTION

Acute graft-versus-host disease (GVHD) is a frequent complication of allogeneic hematopoietic cell transplantation, characterized by skin, intestinal tract, and liver damage [1,2]. After syngeneic [3-5] or autologous stem cell transplantation (ASCT), a GVHD-like syndrome, which is clinically and histologically indistinguishable from allogeneic GVHD, has been described in the skin [3,6-11], intestinal tract [11,12], and liver [11,13]. The frequency of spontaneous skin GVHD after autologous transplantation is 5%-10% [8-10], but rates of 30%-80% have been reported after induction with cyclosporine-based therapy or interleukin-2 (IL-2) [11,14,15]. The etiology of syngeneic and autologous GVHD is not well understood, but there are 2 hypotheses, 1 related to dominance of autoreactive T cells posttransplantation [15] and the other related to the presence of microchimeric (allogeneic) T cells [5].

The probability, risk factors, and morbidity of spontaneously occurring gastrointestinal (GI) GVHD in ASCT recipients remain poorly defined. We previously reported a case series of 8 autograft recipients with histologically proven gastric GVHD and estimated the frequency of this finding to be 4% [12]. Since first recognizing this syndrome in 1995, we have closely followed the course of 681 consecutive patients

who received autologous transplantation after myeloablative therapy, 90 (13%) of whom developed biopsy-proven GI GVHD. Here we report the course and response to treatment for these patients, along with a risk factor analysis for development of this syndrome.

METHODS

Patient Selection

We examined all patients who underwent myeloablative ASCT at our center between July 1995 and July 1999 and evaluated those who experienced persistent GI symptoms, as described later. Review of patient records was carried out under the aegis of a protocol approved by our Institutional Review Board. In this series, no patient received stem cells from a syngeneic donor.

Autologous Transplantation Technique

The majority of patients were conditioned for transplantation with busulfan/melphalan/thioTEPA (BUMELT) [16], total body irradiation/cyclophosphamide/etoposide [17], busulfan/cyclophosphamide, cyclophosphamide/total body irradiation, busulfan/cyclophosphamide/total body irradiation, I^{131}-radiolabeled antibody to CD20 with or without cyclophosphamide/total body irradiation [18], melphalan/thioTEPA, busulfan, or BCNU/etoposide/araC/melphalan (BEAM). Once at our center, all patients received irradiated blood products. Unfortunately, transfusion history outside of our center could not be accurately determined from the medical records provided to us. Fluconazole was given from conditioning therapy until day +75 after stem cell infusion. All patients received prophylactic antibiotics when the absolute neutrophil count (ANC) reached $<5.0 \times 10^9$/L. Patients who were serologically positive for herpes simplex virus (HSV) received prophylactic low-dose acyclovir until engraftment. Patients had weekly cytomegalovirus (CMV) screening with a CMV pp65 antigenemia assay (CMV Brite; Biotest Diagnostics, Denville, NJ) and viral blood testing from day +10 through day +100 after stem cell infusion or until discharge from our system, as described previously [19]. Patients with a positive quantitative CMV antigenemia test ($\geq$5 cells/slide) received antiviral therapy with ganciclovir.

Evaluation of GI Symptoms

Patients were evaluated for development of symptoms of nausea, vomiting, anorexia, dysphagia, heartburn, abdominal pain, and diarrhea up to 100 days post–stem cell infusion. The Gastroenterology/Hepatology Service evaluated patients who exhibited the persistence of 1 or more of these symptoms beyond day +15 post–stem cell infusion. Evaluation consisted of history, physical examination, laboratory tests (serum liver tests, lipase, stool cultures, and microscopy), and an imaging study (computed tomography, x-ray, or ultrasonography) if clinically indicated [12,20]. GI symptoms, such as nausea/vomiting, anorexia, and diarrhea, were graded from 0 (no symptoms) to 3 (severe) [21,22]. When a clear etiology for these symptoms could not be identified, upper GI endoscopy or colonoscopy and mucosal biopsy were performed under conscious sedation using video endoscopy and sterile biopsy forceps for mucosal biopsy, as described previously [22,23]. Endoscopic findings were graded as 0, no abnormality; 1, mild mucosal edema/erythema; 2, moderate mucosal edema/erythema; 3, severe mucosal edema/erythema; 4, focal mucosal ulceration; or 5, diffuse mucosal ulceration. Biopsy specimens taken from 4 sites in the gastric antrum and from any other abnormal-appearing mucosa were placed in B5 formalin for histology (hematoxylin & eosin, methenamine silver, and Brown-Hopps tissue Gram stain), in rapid urease test kits for detection of *Helicobacter pylori* (CLO test; Delta West, Bentley, Australia), and in veal infusion broth for viral culture and tested for fungal pathogens. Viral culture methods included both conventional fibroblast cell culture for microscopic examination of cytopathic alterations and centrifugation cell culture for rapid diagnosis of CMV by indirect immunofluorescence [24].

Diagnosis of Autologous GI GVHD

A diagnosis of GI GVHD was made if the following criteria were met: (1) ongoing GI symptoms, (2) endoscopy or colonoscopy showing abnormal mucosa in the stomach, duodenum, or colon [25]; (3) histological findings in the biopsy specimens showing apoptotic crypt epithelial cells with or without lymphocytic aggregates, or crypt cell dropout with focal lymphocytic infiltration [25-27]; and (3) negative results for viral, bacterial, and fungal pathogens from cultures of stool, mucosal brushings, and biopsy tissue from mucosa involved with GVHD [23,24].

Response of GI Symptoms to Treatment

We defined a *durable response* to treatment as the disappearance of the presenting signs and symptoms of autologous GVHD of the stomach or intestines during the treatment period and the absence of recurrent symptoms after treatment was discontinued. Repeat endoscopy was not carried out in patients whose symptoms ceased. A *partial response* was defined as a decrease in GI signs and symptoms during the treatment period but the return of these symptoms after discontinuation of treatment. *No response* was defined as the persistence of symptoms despite treatment.

Table 1. *Characteristics of 681 Consecutive Recipients of Autologous Stem Cells, by the Presence or Absence of Autologous GVHD Involving the Gastrointestinal Tract*

	No Autologous GVHD (n = 591)	Autologous GVHD Present (n = 90)
Age (mean ± SD, years)	44 ± 15	44 ± 14
Sex (F/M)	301/290 (51%/49%)	66/24 (73%/27%)
Pregnancies (female patients)		
Nulligravid	53 (18%)	13 (20%)
Gravid	215 (71%)	49 (74%)
(Single/multiple births)	(47/168)	(13/36)
Pregnancy history unknown	33	4
Parity (female patients)		
Nulliparous	69 (23%)	20 (30%)
Parous	210 (70%)	44 (67%)
(Single/multiple births)	(35/175)	(9/35)
Parity history unknown	22	2
Breast cancer/hematologic malignancies/other diagnoses	140/345/106 (24%/58%/18%)	39/35/16 (43%/39%/18%)
Busulfan/melphalan/thioTEPA versus other conditioning regimens	324/267 (55%/45%)	65/25 (73%/27%)
Peripheral blood stem cells/bone marrow	560/31 (95%/5%)	90/0 (100%/0%)
CD34 selected stem cells	54 (9%)	10 (11%)
IL-2 therapy posttransplantation	69 (12%)	18 (20%)

Statistical Methods

The probability of GI GVHD was estimated using simple proportions. Logistic regression was used to assess the association of potential risk factors with this probability. Factors examined included sex, diagnosis (breast cancer vs lymphoma, multiple myeloma [MM], or a hematologic malignancy [HM] vs other diagnoses), use of IL-2 therapy, source of stem cells (bone marrow [BM] vs peripheral blood stem cells [PBSCs]), age at transplantation, use of CD34-selected stem cells, conditioning regimen (BUMELT vs radiation-containing vs other), parity (among females), and gravidity (among females). The factors sex and diagnosis are highly correlated and thus were combined, resulting in the following categories: breast cancer; male with lymphoma, MM, or HM; male with other diagnosis; female with lymphoma, MM, or HM; and female with other diagnosis. The impact of GI GVHD on the risk of relapse and mortality was assessed by modeling GVHD as a time-dependent covariate in a Cox regression model. Two-sided *P* values from regression models were derived from the Wald test unless otherwise specified, and no adjustments were made for multiple comparisons.

RESULTS

Patient Characteristics

Most patients were adults, with 54% women (Table 1). Information about previous pregnancies and births was available from 330/367 women, most of whom had been pregnant (264/330; 80%). The diagnoses leading to transplantation were lymphoma (n = 257), breast cancer (n = 179), myeloma (n = 83), other hematologic malignancies (n = 40), other tumors (n = 106), and other conditions (n = 16). The most common conditioning regimen was BUMELT, received by 389/681 patients (57%). Most patients received autologous PBSCs (650/681; 95%); the remainder received BM. Sixty-four patients (9%) received CD34-selected stem cells, and 87 (13%) received IL-2 after transplantation.

Incidence of Autologous GVHD Involving the Stomach and Intestinal Tract

Of 681 patients who underwent transplantation during the 4-year period, 109 patients (16%) had persistent GI symptoms after transplantation and underwent intestinal endoscopy with mucosal biopsy. A diagnosis of biopsy-proven GVHD was made in 90 patients (13%). Of note, skin lesions consistent with GVHD were present in only 9 patients (1%), and no patient in this series had evidence of hepatic GVHD.

Symptoms of GI GVHD

Persistent nausea and vomiting were the most common symptoms of GVHD, affecting 81/90 patients (90%) (Table 2). Two patients presented with only anorexia, 3 patients with only diarrhea, 3 patients with only dysphagia, and 1 patient with only abdominal pain. In 41 of the 81 patients, anorexia accompanied nausea and vomiting, and in 33, diarrhea was also present. The severity of nausea, vomiting, or anorexia was generally moderate, but some patients were disabled by incessant vomiting and profound anorexia. Only 40% of all patients with a diagnosis of GVHD had diarrhea, which was generally mild. There were no cases of severe diarrhea. The mean time to onset of GI symptoms was day +15 post–stem cell infusion, but some patients developed symptoms within days of completing conditioning therapy that did not resolve with the passage of time. However, most patients

Table 2. *Onset and Severity of the Symptoms of Gastrointestinal GVHD in 90 Patients*

	Number of Patients	Mean Day Post–Stem Cell Infusion to Onset of Symptoms	Severity of Symptoms (Range, 0-3)
Nausea/vomiting with or without anorexia or diarrhea	81	14.9 ± 18.4 (range, 0-77)	1.6 ± 0.6 (range, 1-3)
Anorexia with or without nausea/vomiting or diarrhea	43	14.3 ± 19.3 (range, 0-77)	1.8 ± 0.6 (range, 1-3)
Diarrhea with or without anorexia or nausea/vomiting	36	14.5 ± 16.1 (range, 0-63)	1.5 ± 0.5 (range, 1-2)

experienced resolution of conditioning therapy–related symptoms but later developed anorexia, nausea, vomiting, or diarrhea, with onset of symptoms as late as day +77 post–stem cell infusion.

Endoscopic Findings

The mean time to first diagnostic endoscopy was day +42 ± 17 after stem cell infusion (range, day +18-day +98), with upper endoscopy performed in 87 patients and colonoscopy performed in 3 patients. Because the mean time to endoscopy or colonoscopy was 42 days after stem cell infusion, we believe that adequate time was allowed for the patients to recover from acute conditioning toxicity before the procedure was done to assess for GVHD. Endoscopic abnormalities were found more often in the stomach, where 82/87 patients (94%) had mucosal abnormalities, than in the duodenum, where 47/86 patients (55%) had abnormal-appearing mucosa. Colonic mucosal abnormalities were seen in 2 of 3 patients. Most of the mucosal abnormalities consisted of grades 1–2 (mild to moderate) mucosal edema and erythema; however, some patients had severe grade 3 edema and erythema in the gastric (n = 12) or duodenal mucosa (n = 5). One patient had grade 4 focal mucosal ulceration in the stomach.

Histological Findings

The most common histological finding in the mucosal biopsy specimens was apoptosis of epithelial cells in gastric or colonic crypts, found in 82/90 patients (91%). Crypt cell apoptosis was frequently found in association with focal lymphoid aggregates (61/90; 68%), but was also seen as an isolated finding (21/90; 23%). Eight patients (9%) exhibited histological evidence of crypt cell dropout and focal lymphocyte aggregates.

Response to Treatment

A total of 82 patients were treated with corticosteroids (70 with prednisone; 9 with oral beclomethasone dipropionate at 8 mg, given in 4 divided doses in an emulsion formulation; and 3 with oral beclomethasone dipropionate for a few days followed by prednisone). The initial dose of oral prednisone in 67/73 patients (92%) was 1 mg/kg/day for 10-4 days, followed by a slow tapering of the dose. A prednisone dose of 0.5 mg/kg/day was given to 1 patient, and a dose of 2 mg/kg/day was given to 5 patients. The mean day for initiation of treatment was day +45 ± 19 post–stem cell infusion (range, day +22-day +103).

Durable Responses. Sixty-five of 82 treated patients (79%) had a cessation of all GI symptoms that was sustained after corticosteroid therapy was discontinued. There was only a short interval from the start of therapy to improvement in symptoms (median, 4 days; range, 1-12 days). Of the 65 patients with a durable response to therapy, 56 had been treated with prednisone, 6 with oral beclomethasone dipropionate, and 3 with a few days of oral beclomethasone dipropionate followed by prednisone. The mean full duration of therapy, including time to complete a tapering of the prednisone dose to avoid adrenal insufficiency, was 39 ± 17 days (range, 3-95 days). There were no major complications of corticosteroid therapy, although infection at baseline in 2 patients led to management problems. One patient developed oral HSV infection; treatment with acyclovir was started 5 days before the initiation of a 2-week course of prednisone therapy. One patient developed parainfluenza pneumonia and respiratory insufficiency 5 days before starting prednisone therapy and died of respiratory failure 2 weeks later.

Partial Responses. Seventeen patients experienced improvement in symptoms in response to initial therapy (12 treated with prednisone 1 mg/kg/day for 10-14 days, followed by a tapering of the dose; 2 treated with prednisone 2 mg/kg/day; and 3 treated with oral beclomethasone dipropionate emulsion). The median duration of initial therapy was 31 days (range, 10-51 days). But all patients in this latter cohort experienced recurrent nausea, vomiting, or anorexia after corticosteroid treatment was discontinued. All patients underwent repeat endoscopy, at a median of 19 days from the final day of corticosteroid treatment (range, 4-49 days). Gastric mucosal biopsy specimens exhibited epithelial cell apoptoses in 14 of the 17 patients, with focal lymphoid aggregates found in the remaining 3 patients. Retreatment with corticosteroids was initiated in the former group of 14

Table 3. *Univariate Logistic Regression Models for Development of Autologous GVHD*

	OR	95% CI	*P* Value
Age (years)*	1.03	0.89-1.21	.70
Sex			
Male	1	—	—
Female	2.65	1.62-4.34	.0001
Parity (females only)			
Nulliparous	1	—	—
Parous	0.72	0.40-1.31	.28
Pregnancy (females only)			
Nulligravid	1	—	—
Gravid	0.93	0.47-1.84	.83
Diagnosis			
Other diagnoses	1	—	—
Breast cancer	1.85	0.98-3.48	.06
Hematologic malignancy	0.67	0.36-1.26	.22
Stem cell source			
Bone marrow	1	—	—
Peripheral blood	∞	—	.003†
Conditioning regimen			
BUMELTT	1	—	—
Other	2.28	1.39-3.74	.001
CD 34 selection of infused stem cells			
Unselected	1	—	—
Selected	0.79	0.39-1.58	.50
Interleukin-2 therapy			
No	1	—	—
Yes	1.89	1.07-3.36	.03

*Modeled as a continuous linear variable, with odds ratio presented as increase in odds for each 10-year increase in age.

†*P* value obtained from likelihood ratio test.

patients (prednisone starting doses of 1 mg/kg/day in 12, 1.5 mg/kg/day in 1, and 2 mg/kg/day in 1). The median time to improvement in symptoms was 4 days (range, 2-14 days). All 14 patients had durable responses to this second course of corticosteroid therapy. After a median treatment period of 34 days (range, 15-64 days), there were no recurrences of symptoms after discontinuation of prednisone. One patient had treatment deferred because of reactivation of hepatitis B virus, but after a third endoscopy showed autologous GVHD, this patient was treated with prednisone, 1 mg/kg/day, and achieved a durable response. Two patients whose recurrent symptoms were mild were never retreated.

Untreated Patients. Eight patients received no treatment despite gastric mucosal abnormalities on endoscopy and abnormal histology consistent with autologous GVHD. Three patients presented with dysphagia as their only GI symptom; 2 of these patients improved after treatment for reflux esophagitis, and 1 improved after ganciclovir therapy was begun for CMV esophagitis. One nauseated patient had esophageal CMV and gastric GVHD diagnosed at endoscopy and improved after ganciclovir therapy. Three patients with mild symptoms improved without therapy. One patient with persistent, moderately severe nausea, vomiting, anorexia, and diarrhea underwent endoscopy 3 times over 79 days of observation, and each endoscopy showed GVHD, which was never treated.

Analysis of Risk Factors for the Development of Autologous GI GVHD

In a univariate analysis, potential risk factors, including age, sex, pregnancy history, disease type, stem cell source, transplantation conditioning regimen, PBSC graft manipulation with or without CD34 selection, and addition of immunotherapy, were assessed. The findings are summarized in Table 3.

All patients diagnosed with breast cancer were female, so the impact of sex was examined in the patients with diagnoses other than breast cancer. The impact of sex appeared to differ in those patients with a hematologic malignancy compared with those with other diagnoses (excluding breast cancer) ($P = .04$), so sex and diagnosis were combined into a single variable with categories as described earlier in Statistical Methods. Table 4 summarizes results from a multivariable logistic regression model, including the use of this combined factor.

The odds of GI GVHD were 3.27 times higher in females with breast cancer compared to males with a hematologic malignancy (95% confidence interval = 1.72-6.22; $P = .0003$). Women with a diagnosis of hematologic malignancy also had a higher risk than men with a hematologic malignancy (odds ratio [OR] = 2.95; 95% confidence interval [CI] = 1.44-6.01; $P = .003$), suggesting a sex effect in patients with such a diagnosis. Such an effect does not appear to exist among patients with other diagnoses, however; the risk of GI GVHD is actually lower in females than in males in this group. The use of BUMELT is suggestively associated with an increased probability of

Table 4. *A Multivariable Logistic Regression Model for Development of Gastrointestinal Autologous GVHD*

Factor	OR	95% CI	*P* Value
Sex/Diagnosis			
Male/hematologic malignancy	1	—	—
Female/breast cancer	3.27	1.72-6.22	.0003
Female/hematologic malignancy	2.95	1.44-6.01	.003
Male/other diagnoses	2.82	1.13-7.09	.03
Female/other diagnoses	2.12	0.86-5.24	.10
Interleukin-2 therapy			
No	1	—	—
Yes	1.79	0.98-3.26	.06
Conditioning regimen			
BUMELTT	1	—	—
Other	1.69	0.98-2.89	.06
Stem cell source			
Bone marrow	1	—	—
Peripheral blood	∞	—	.02*

**P* value obtained from likelihood ratio test.

autologous GI GVHD compared with all other regimens, and although the majority (87%) of patients with breast cancer received BUMELT, the impact of BUMELT was roughly the same across the 3 disease categories ($P = .34$ for breast cancer vs hematologic malignancy; $P = .76$ for hematologic malignancy vs other diagnoses). The use of PBSC and IL-2 therapy were also associated with suggestively increased risk of GI GVHD compared with the use of BM and no immunotherapy, respectively. Among female patients, parous women were less likely to develop GI GVHD than nulliparous women after adjusting for the relevant factors listed in Table 4, but the difference was not statistically significant (OR = 0.60; $P = .13$; likelihood ratio test).

Effect of Autologous GI GVHD on Mortality

The hazard of mortality in patients with autologous GI GVHD was lower than that in patients without GVHD, but the difference was not statistically significant (hazard ratio = 0.89; 95% CI = 0.64-1.22; $P = .46$). The magnitude of association between GVHD and the hazard of mortality was not suggestively different across disease groups (data not shown).

DISCUSSION

The major findings of this study can be summarized as follows:

1. *Autologous GVHD involving the GI tract developed in 13% of the patients and was responsive to a single course of prednisone therapy in most of these patients.*
2. *Both female sex and diagnosis are associated with the development of autologous GI GVHD, with the impact of sex apparently depending on diagnosis.*

The clinical presentation of autologous GI GVHD was identical to that of recipients of allogeneic hematopoietic stem cells who develop nausea, vomiting, and anorexia [21,22,27,28]. Diarrhea, usually mild to moderate in severity and almost always associated with nausea or anorexia, occurred in 40% of the patients. The endoscopic appearance and histology of the mucosa in the stomach and duodenum were identical to those of allogeneic GVHD. (Color plates of endoscopic photographs [25] and gastric mucosal histology [12] have been published previously.) A short treatment course of prednisone resulted in complete, durable resolution of symptoms in 79% of the treated patients. An additional 18% of patients were retreated and had complete, durable responses to another course of corticosteroid therapy. In our previous report of patients undergoing autologous hematopoietic cell transplantation, we made a diagnosis of autologous GI GVHD in 4% of cases [12]. The 13% incidence in the current series reflects an increased awareness of this syndrome among our clinical staff, along with an imperative to discover a cause for persistent GI symptoms before patients were returned to the care of their referring physicians. Our current practice is to consider the diagnosis of autologous GI GVHD in patients whose appetite is not improving by day +15-20 after stem cell infusion, and to perform endoscopy in all patients whose intestinal symptoms persist beyond day +25 to evaluate for GVHD, enteric infection by bacteria and viruses (especially CMV), or persistence of significant conditioning-related enterocolitis [23,24,29].

In our study, all patients who developed autologous GVHD received granulocyte colony-stimulating factor (G-CSF)-mobilized PBSC grafts. These grafts are known to have 10 times more T cells and 50 times more monocytes than BM [30]. Conditioning with BUMELT was also suggestively associated with autologous GI GVHD (adjusted OR = 1.69; $P = .06$), and this effect did not appear to be due solely to its use among patients with breast cancer. This regimen is known to cause significant GI toxicity [16,31-33]. In our study of syngeneic graft recipients, we also observed an association between GVHD and BUMELT conditioning therapy ($P < .01$) [5]. We speculate that GI mucosal damage from this preparative regimen increases the risk of autologous GI GVHD. Activated cytotoxic T cells cause further damage directly to the GI mucosa. Because early after transplantation, thymic function is poor and older patients are not likely to have much thymus function even before transplantation, thymic dysfunction makes our patients more susceptible to developing autoreactive immune cells. In addition, immune regulatory cells that control the activity of autoreactive cells are decreased in the periphery by the conditioning transplantation regimen. This decrease in immune regulatory cells and subsequent decrease in immune regulatory cytokines, such as transforming growth factor β-1, may increase the ability to develop autoreactive cells. Thus, all of these changes contribute to tipping the balance toward being able to generate autologous GVHD.

In patients with hematologic malignancies or breast cancer, a graft-versus-tumor effect with decreased relapse rates and survival benefit has been reported for patients who develop cyclosporine-induced autologous GVHD [34-40]. Both animal models and clinical trials suggest that the use of cyclosporine results in a permissive environment that maximizes early after autologous transplantation the development of autoreactive cells. Autologous GVHD results not only from the failure to inhibit the deletion of autoreactive T cells by the thymus, but also from the elimination of peripheral immunoregulatory cells [15]. We could not demonstrate a survival benefit from the mainly spontaneous GI GVHD in our patient cohort as a whole or in any single disease cohort. However, controversy exists as to which types of immune cells are most important for

a graft-versus-tumor effect. In cyclosporine-induced models of GVHD, perforin/grazyme B-containing cells are preferentially increased over FAS/FAS ligand–containing cells, which are increased only marginally [41]. In allogeneic GVHD disease, initially FAS/FAS ligand–-containing cells were felt to be more important in tumor control [42]. Other studies indicate that perforin pathways are more important than FAS/FAS ligand pathways in differentiating the cells that cause allogeneic GVHD from those that have a graft-versus-tumor effect [43]. Because we do not know which types of immune cells are associated with the mainly spontaneous, non–cyclosporine-induced autologous GI GVHD reported here, it is possible that the cells mediating predominately autologous GI GVHD are not those that contribute to a graft-versus-tumor effect.

An interesting finding in this series is the differential association of autologous GI GVHD with female sex according to diagnosis. Females with breast cancer were more likely to develop GI GVHD compared with males with hematologic malignancy, but it is not possible to ascribe this association to sex or diagnosis. A sex effect was seen in patients with a hematologic malignancy, however, where females had nearly 3 times the odds of GVHD compared to males. Such an effect was not seen in patients with other diagnoses, however. Females with breast cancer and those with a hematologic malignancy had an increased risk of GI GVHD compared with females with other diagnoses, but neither association was statistically significant (OR = 1.54, $P = .32$ for breast cancer; OR = 1.39, $P = .47$ for hematologic malignancy) One hypothesis to explain the association of female sex with GVHD is a biologically plausible one: Pretransplantation hematopoietic microchimerism results in the transplantation of both autologous and allogeneic immune cells, and these allogeneic cells lead to GVHD. Microchimerism can be established in 2 ways. First, women have a higher frequency of microchimerism than men, as a result of fetal cells in the maternal circulation during and for decades after pregnancy [44]. Recently, male DNA has been frequently found in the G-CSF–mobilized apheresis PBSC product from normal female donors [44,45]. Donor parity is reportedly associated with syngeneic and allogeneic GVHD, thought to be related to allosensitization of maternal T cells to fetal antigens during pregnancy, thus priming these cells to recognize similar antigens in recipients of hematopoietic cells from parous donors [46-48]. Our group has recently reported that syngeneic GVHD occurs more frequently when the donor is a parous female (32%) rather than a nulliparous female (9%) or a male (13%) ($P = .03$). In addition, syngeneic GVHD is associated with the parity of the syngeneic recipient; a parous female (31%) is more likely to develop GVHD than a nulliparous female (7%) or a male (13%) ($P = .02$) [5]. In the current analysis, we were not able to show a statistically significant association between parity or gravidity of our female patients and the risk of developing autologous GI GVHD.

The second mechanism for microchimerism is through blood transfusions. However, we were unable to provide data on this alternate mechanism by which our patients may have become microchimeric, that is, through transfer of allogeneic cells through transfusion of unirrradiated blood products. Before receiving myeloablative autologous transplantation, a patient often undergos many courses of therapy, and may not have been immunologically competent enough to reject allogeneic cells transfused in unirrradiated blood products. Microchimerism acquired through blood product transfusion applies equally to all patients in our study cohort, including both men and nulliparous women. We could not test this hypothesis, because we lacked detailed history of blood transfusions before the patients entered our system and did not test patients' blood or stem cell products using molecular methods to detect microchimerism.

If the microchimerism hypothesis of GVHD in autologous graft recipients is correct, then this disorder should be more properly called allogeneic GVHD. Unlike the situation in allogeneic hematopoietic cell transplantation, most of our patients responded to a short course of corticosteroid therapy, suggesting that HLA-disparate cells or autoreactive cells were easily rejected or destroyed.

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REFERENCES

1. Sullivan KM. Graft-versus-host disease. In: Blume KG, Forman SJ, Appelbaum FR, eds. *Thomas' Hematopoietic Cell Transplantation*. 3rd ed. Malden, MA: Blackwell Scientific Publications; 2004.
2. Martin PJ, McDonald GB, Sanders JE, et al. Increasingly frequent diagnosis of acute graft-versus-host disease after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2004;10:320-327.
3. Gluckman E, Devergie A, Sohier J, et al. Graft-versus-host disease in recipients of syngeneic bone marrow. *Lancet*. 1980; 1:253-254.
4. Einsele H, Ehninger G, Hebart H, et al. Incidence of local CMV infection and acute intestinal GVHD in marrow transplant recipients with severe diarrhoea. *Bone Marrow Transplant*. 1994;14:955-963.
5. Adams KM, Holmberg LA, Leisenring W, et al. Risk factors for syngeneic graft-versus-host disease after adult hematopoietic cell transplantation. *Blood*. 2004;104:1894-1897.
6. Einsele H, Ehninger G, Schneider EM, et al. High frequency of

graft-versus-host-like syndromes following syngeneic bone marrow transplantation. *Transplantation*. 1988;45:579-585.
7. Thein SL, Goldman JM, Galton DAG. Acute "graft-versus-host disease" after autografting for chronic granulocytic leukemia in transformation. *Ann Intern Med*. 1981;94:210-211.
8. Hood AF, Vogelsang GB, Black LP, et al. Acute graft-versus-host disease: development following autologous and syngeneic bone marrow transplantation. *Arch Dermatol*. 1987;123:745-750.
9. Jones RJ, Vogelsang GB, Hess AD, et al. Induction of graft-versus-host disease after autologous bone marrow transplantation. *Lancet*. 1989;i:754-757.
10. Carella AM, Gaozza E, Congiu A, et al. Cyclosporine-induced graft-versus-host disease after autologous bone marrow transplantation in hematological malignancies. *Ann Hematol*. 1991; 62:156-159.
11. Baron F, Gothot A, Salmon JP, et al. Clinical course and predictive factors for cyclosporin-induced autologous graft-versus-host disease after autologous haematopoietic stem cell transplantation. *Br J Haematol*. 2000;111:745-753.
12. Tzung S-P, Hackman RC, Hockenbery DM, et al. Lymphocytic gastritis resembling graft-vs.-host disease following autologous hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 1998;4:43-48.
13. Saunders MD, Shulman HM, Murakami CS, et al. Bile duct apoptosis and cholestasis resembling acute graft-versus-host disease after autologous hematopoietic cell transplantation. *Am J Surg Pathol*. 2000;24:1004-1008.
14. Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. *N Engl J Med*. 2000;343:327-331.
15. Hess A, Jones R. Autologous graft-vs.-host disease. In: Blume K, Forman SJ, Appelbaum F, eds. *Thomas' Hematopoietic Cell Transplantation*. 3rd ed. Malden, MA: Blackwell Scientific Publishing; 2004. p. 405-413.
16. Schiffman KS, Bensinger WI, Appelbaum FR, et al. Phase II study of high-dose busulfan, melphalan and thiotepa with autologous peripheral blood stem cell support in patients with malignant disease. *Bone Marrow Transplant*. 1996;17: 943-950.
17. Brunvand MW, Bensinger WI, Soll E, et al. High-dose fractionated total-body irradiation, etoposide and cyclophosphamide for treatment of malignant lymphoma: comparison of autologous bone marrow and peripheral blood stem cells. *Bone Marrow Transplant*. 1996;18:131-141.
18. Gopal AK, Rajendran JG, Petersdorf SH, et al. High-dose chemo-radioimmunotherapy with autologous stem cell support for relapsed mantle cell lymphoma. *Blood*. 2002;99: 3158-3162.
19. Boeckh M, Stevens-Ayers T, Bowden RA. Cytomegalovirus pp65 antigenemia after autologous marrow and peripheral blood stem cell transplantation. *J Infect Dis*. 1996;174:907-912.
20. Strasser SI, McDonald GB. Gastrointestinal and hepatic complications. In: Blume KG, Forman SJ, Appelbaum FR, eds. *Thomas' Hematopoietic Cell Transplantation*. 3rd ed. Malden, MA: Blackwell Scientific Publications; 2004. p. 769-810.
21. Spencer GD, Hackman RC, McDonald GB, et al. A prospective study of unexplained nausea and vomiting after marrow transplantation. *Transplantation*. 1986;42:602-607.
22. Wu D, Hockenbery DM, Brentnall TA, et al. Persistent nausea and anorexia after marrow transplantation: a prospective study of 78 patients. *Transplantation*. 1998;66:1319-1324.
23. Cox GJ, Matsui SM, Lo RS, et al. Etiology and outcome of diarrhea after marrow transplantation: a prospective study. *Gastroenterology*. 1994;107:1398-1407.
24. Hackman RC, Wolford JL, Gleaves CA, et al. Recognition and rapid diagnosis of upper gastrointestinal cytomegalovirus infection in marrow transplant recipients: a comparison of seven virologic methods. *Transplantation*. 1994;57:231-237.
25. Ponec RJ, Hackman RC, McDonald GB. Endoscopic and histologic diagnosis of intestinal graft-vs.-host disease after marrow transplantation. *Gastrointest Endosc*. 1999;49:612-621.
26. Snover DC, Weisdorf SA, Vercellotti GM, et al. A histopathologic study of gastric and small intestine graft-versus-host disease following allogeneic bone marrow transplantation. *Hum Pathol*. 1985;16:387-392.
27. Washington K, Bentley RC, Green A, et al. Gastric graft-versus-host disease: a blinded histologic study. *Am J Surg Pathol*. 1997;21:1037-1046.
28. Weisdorf DJ, Snover DC, Haake R, et al. Acute upper gastrointestinal graft-versus-host disease: clinical significance and response to immunosuppressive therapy. *Blood*. 1990;76: 624-629.
29. Holmberg LA, Boeckh M, Hooper H, et al. Increased incidence of cytomegalovirus disease after autologous CD34-selected peripheral blood stem cell transplantation. *Blood*. 1999;94:4029-4035.
30. Kusnierz-Glaz CR, Still BJ, Amano M, et al. Granulocyte colony-stimulating factor-induced comobilization of CD4− CD8− T cells and hematopoietic progenitor cells (CD34+) in the blood of normal donors. *Blood*. 1997;89:2586-2595.
31. Gutierrez-Delgado F, Holmberg L, Hooper H, et al. Autologous stem cell transplantation for Hodgkin's disease: busulfan, melphalan and thiotepa compared to a radiation-based regimen. *Bone Marrow Transplant*. 2003;32:279-285.
32. Holmberg LA, Demirer T, Rowley S, et al. High-dose busulfan, melphalan and thiotepa followed by autologous peripheral blood stem cell (PBSC) rescue in patients with advanced stage III/IV ovarian cancer. *Bone Marrow Transplant*. 1998; 22:651-659.
33. Bensinger WI, Schiffman KS, Holmberg L, et al. High-dose busulfan, melphalan, thiotepa and peripheral blood stem cell infusion for the treatment of metastatic breast cancer. *Bone Marrow Transplant*. 1997;19:1183-1189.
34. Marin GH, Menna ME, Bergna MI, et. Induction of antitumor activity following autologous stem cell transplantation: immunotherapeutic implications. *Transplant Proc*. 2001;33: 2004-2007.
35. Kennedy MJ, Vogelsang GB, Beveridge RA, et al. Phase 1 trial of intravenous cyclosporine to induce graft-versus-host disease in women undergoing autologous bone marrow transplantation for breast cancer. *J Clin Oncol*. 1993;11:478-484.
36. Gryn J, Johnson E, Goldman N, et al. The treatment of relapsed or refractory intermediate-grade non-Hodgkin's lymphoma with autologous bone marrow transplantation followed by cyclosporine and interferon. *Bone Marrow Transplant*. 1997; 19:221-226.
37. Yeager AM, Vogelsang GB, Jones RJ, et al. Induction of cutaneous graft-versus-host disease by administration of cyclosporine to patients undergoing autologous bone marrow transplantation. *Blood*. 1992;79:3031-3035.
38. Tricot G, Vesole DH, Jagannath S, et al. Graft-versus-myeloma effect: proof of principle. *Blood*. 1996;87:1196-1198.
39. Verdonck LF, Lokhorst HM, Dekker AW, et al. Graft-versus-myeloma effect in two cases. *Lancet*. 1996;347:800-801.

40. Vogelsang G, Bitton R, Piantadosi S, et al. Immune modulation in autologous bone marrow transplantation: cyclosporine and gamma-interferon trial. *Bone Marrow Transplant*. 1999;24:637-640.
41. Miura Y, Thoburn CJ, Bright EC, et al. Cytolytic effector mechanisms and gene expression in autologous graft-versus-host disease: distinct roles of perforin and Fas ligand. *Biol Blood Marrow Transplant*. 2004;10:156-170.
42. Schmaltz C, Alpdogan O, Horndasch KJ, et al. Differential use of Fas ligand and perforin cytotoxic pathways by donor T cells in graft-versus-host disease and graft-versus-leukemia effect. *Blood*. 2001;97:2886-2895.
43. Reddy P, Teshima T, Kukuruga M, et al. Interleukin-18 regulates acute graft-versus-host disease by enhancing Fas-mediated donor T cell apoptosis. *J Exp Med*. 2001;194:1433-1440.
44. Adams KM, Nelson JL. Microchimerism: an investigative frontier in autoimmunity and transplantation. *JAMA*. 2004;291:1127-1131.
45. Adams KM, Lambert NC, Heimfeld S, et al. Male DNA in female donor apheresis and CD34-enriched products. *Blood*. 2003;102:3845-3847.
46. Kollman C, Howe CW, Anasetti C, et al. Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood*. 2001;98:2043-2051.
47. Verdijk RM, Kloosterman A, Pool J, et al. Pregnancy induces minor histocompatibility antigen-specific cytotoxic T cells: implications for stem cell transplantation and immunotherapy. *Blood*. 2004;103:1961-1964.
48. Flowers ME, Pepe MS, Longton G, et al. Previous donor pregnancy as a risk factor for acute graft-versus-host disease in patients with aplastic anaemia treated by allogeneic marrow transplantation. *Br J Haematol*. 1990;74:492-496.