

ORIGINAL ARTICLE

Female genital tract graft-versus-host disease: incidence, risk factors and recommendations for management

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Female genital tract graft-versus-host disease (GVHD) is an under-recognized complication of allogeneic stem cell transplantation impacting on quality of life. We describe a prospective surveillance programme for female genital GVHD to better characterize incidence, risk factors and clinical features and the impact of a structured intervention policy. A retrospective audit was conducted on the medical records of all female transplant recipients surviving at least 6 months at a single centre over a 5-year period. Patients commenced topical vaginal oestrogen early post transplant with hormone replacement as appropriate for age, prior menopausal status and comorbidities. A genital tract management programme included regular gynaecological review and self-maintenance of vaginal capacity by dilator or intercourse. The incidence of genital GVHD was 35% (95% confidence interval (CI) (25, 50%)) at 1 year and 49% (95% CI (36, 63%)) at 2 years. Topical therapy was effective in most cases; no patient required surgical intervention to divide vaginal adhesions. The main risk factor was stem cell source with peripheral blood progenitor cells posing a higher risk than marrow (hazard ratio = 3.07 (1.22, 7.73), $P=0.017$). Extensive GVHD in other organs was a common association. We conclude that female genital GVHD is common, and early detection and commencement of topical immunosuppression with dilator use appears to be highly effective at preventing progression. *Bone Marrow Transplantation* (2006) 38, 567–572. doi:10.1038/sj.bmt.1705487; published online 4 September 2006

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Introduction

Female genital tract graft-versus-host disease (GVHD) is an under-recognized complication of allogeneic stem cell

transplantation (SCT), reported to occur in a quarter of long-term female survivors.¹ Clinical features range from vulval or vaginal irritation, discharge and ulceration to vaginal stenosis. Symptoms of mild genital GVHD overlap with those of genital tract atrophy caused by premature ovarian failure commonly associated with myeloablative therapies. The majority of published case reports are skewed to the advanced stenosing lesions requiring surgical therapy.^{2,3} As genital tract GVHD impacts on quality of life, sexual function and relationship dynamics, early recognition and treatment are an important part of post transplant management. In the late-1990s, we initiated a programme to standardize detection, grading of severity and management of female genital tract GVHD. This paper describes the analysis of our results over a 5-year period, and proposes a surveillance and management programme for routine incorporation into post transplant care.

Patients and methods

Eligibility for inclusion in analysis

Female recipients of an allogeneic stem cell transplant at the Royal Melbourne Hospital from May 1999 to July 2004 were eligible if they engrafted with donor cells, were alive and in remission for at least 6 months post transplant, and had adequate local follow-up. Patients who relapsed and subsequently received chemotherapy or donor leucocyte infusions after 6 months were censored at the time of relapse.

Assessment of genital tract manifestations

Data were collected by retrospective audit of medical records during the study period. The date of onset of genital tract GVHD was taken as the date of the gynaecological review at which the diagnosis was confirmed. All women were assessed by a single gynaecological team with an interest in post transplant care. Standard gynaecological management was similar to recently published guidelines⁴ and included:

1. Pre-transplant patient counselling regarding the early recognition of symptoms of genital tract GVHD and oestrogen deficiency.

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2. Early commencement of post transplant vaginal topical oestrogen (oestriol 0.1% vaginal cream, 0.5 g per vagina twice weekly) in all patients.
3. Early commencement of systemic oestrogen and progestogen replacement as appropriate for age, prior menopausal status based on gonadotrophin levels and co-morbidities.
4. Consideration of systemic androgen replacement for androgen deficiency symptoms (such as tiredness and poor libido associated with low serum testosterone levels) from 6 months post transplant.
5. Early patient commencement of genital tract self-surveillance by vaginal self-examination or dilator passage, in the absence of regular sexual intercourse.
6. Regular gynaecological symptom review and pelvic examination from 3 months post transplant for assessment of genital tract GVHD using a standardized 'in house' severity grading, as detailed in Table 1, and similar to that reported previously.¹ Features noted were vulvo-vaginal burning or irritation, watery vaginal discharge and dyspareunia, and patchy, erythematous or desquamative inflammation of the vulva or vagina, loss of vaginal elasticity, fibrinous vaginal adhesions, circumferential fibrous vaginal banding and reduction in vaginal capacity. The severity grading was applied at each gynaecological review.
7. Annual cervical cytology.

GVHD prophylaxis and assessment

The day of transplant was day 0. GVHD prophylaxis consisted of methotrexate 15 mg/m² on day 1 and 10 mg/m² on days 3, 6 and 11 and cyclosporine in all patients. Intravenous cyclosporine was commenced on day -1 at 3 mg/kg daily, except in fludarabine-melphalan conditioning where a loading dose of 5 mg/kg was given for 3 days and then continued at 3 mg/kg daily. From October 2000, additional glucocorticosteroid prophylaxis starting at day 14 was used according to previously reported guidelines in patients at standard risk of disease relapse.⁵ No *in vivo* or *in vitro* T-cell depletion was used. Assessment of engraftment⁶ and acute or chronic GVHD in organs other than the genital tract^{7,8} was performed according to conventional criteria. Data on ocular GVHD were not routinely recorded for this cohort of patients, and details were

Table 1 Severity grading of genital tract GVHD

Severity grading	Description
Mild	Genital tract discomfort and or discharge Inflammatory redness of the vaginal and or vulval mucosa
Moderate	Desquamative or erosive inflammatory change of the genital mucosa Fibrinous exudates Reduction in vaginal elasticity
Severe	Vaginal adhesions Concentric fibrous banding of the vagina Reduction in vaginal capacity Vaginal stenosis or occlusion

Abbreviation: GVHD = graft-versus-host disease.

included only if symptoms were documented or therapy was required.

Management of genital tract GVHD

Genital tract GVHD was managed as follows:

1. Confirmation of compliance with systemic and topical hormone therapy, to eliminate any concomitant oestrogen deficiency (atrophic) component.
2. Topical genital tract glucocorticosteroid therapy consisting of high vaginal application of hydrocortisone acetate 100 mg/g mucoadherent rectal foam 1 g daily for 4–6 weeks, followed by serial reduction in dose frequency according to response.
3. If the response to topical steroid was inadequate, topical genital tract cyclosporine therapy was initiated. This consisted of cyclosporine oral solution 100 mg/ml, 1 ml in 20 ml normal saline high vaginal installation for 15 min daily for 4–6 weeks, followed by serial reduction in dose frequency according to response.
4. Prophylactic vaginal self-examination or dilator insertion (or intercourse) twice weekly to maintain vaginal capacity and prevent the formation of fibrinous genital tract adhesions and vaginal stenosis.
5. Therapeutic vaginal dilator insertion once to twice daily was instituted for established vaginal narrowing. Commercially available vaginal dilators with a tapered design facilitated use in cases of tight vaginal stenosis. Once any tight stenosis was overcome, tapered dilators were replaced by round-end dilators to overcome persistent stenosis of the upper vagina. When an adequate vaginal capacity was achieved, this was maintained by regular intercourse or prophylactic twice-weekly vaginal dilator insertion.

Assessment of response

Genital tract GVHD response was categorized as:

1. 'Completely resolved' when an absence of active inflammatory change and normal vaginal capacity was noted on examination.
2. 'Improved' when a reduction in inflammatory changes and improved vaginal capacity were documented.
3. 'Stabilised' when a lack of active inflammatory change and no progression in the severity of vaginal stenosis as determined by vaginal capacity were documented.
4. 'Progressive' when active inflammatory change persisted or progressed and/or there was progressive reduction in the vaginal capacity owing to stenosis.

Statistical analysis

Potential prognostic variables were tested using a Cox proportional hazards model. These included donor age and sex, stem cell source, conditioning regimen, use of steroid GVHD prophylaxis in addition to cyclosporine and methotrexate and donor human leucocyte antigen match (sibling versus unrelated). Failure time was the time at which the patient was known to first develop genital tract GVHD of any severity. Subjects who did not develop genital tract GVHD while under observation were included as censored observations. Censoring occurred at the date of

last gynaecological review, time of disease relapse or death. Data are represented in terms of the Kaplan–Meier failure function (the inverse of the survivor function). Kaplan–Meier estimates and hazard ratios (HRs) are expressed with 95% confidence limits and HRs >1 indicate a greater probability of genital tract GVHD at any given time. Underlying disease was grouped as myeloproliferative disorders, all acute leukaemia or myelodysplasia and all lymphoproliferative disorders and multiple myeloma. Conditioning regimens were categorized as: (i) myeloablative (fludarabine–melphalan, busulphan–cyclophosphamide with or without etoposide and total body irradiation with cyclophosphamide or etoposide) and (ii) non-myeloablative (fludarabine–cyclophosphamide). Disease type and conditioning regimen were assessed as multi-level categorical variables, and tested using the likelihood ratio test. Model assumptions were assessed graphically and with scaled Schoenfeld residuals, and found to be valid. Stem cell source was the only explanatory variable included in the final model; hence, the reported effects of each remaining variable were adjusted for this.

To assess the relationship between genital tract GVHD and other organ chronic GVHD (i.e. whether other organ sites might be prognostic of genital tract GVHD), patients were classified according to whether or not they developed gut, skin, liver or oral chronic GVHD within the first 12 months. Eight patients had fewer than 12 months of follow-up, so were excluded from this analysis. Chronic GVHD at other organ sites was not analysed for association owing to small sample size. Odds ratios (ORs) for developing genital tract GVHD were calculated for each organ site and assessed using Fisher’s exact test. Statistical analysis was performed using Stata Version 7.0 (StataCorp 2001, Stata Statistical Software: Release 7.0, Stata Corporation, College Station, TX, USA).

Approvals for this study were provided by the Ethics and Research committees of the Royal Women’s and Royal Melbourne Hospitals for access to patient records.

Results

Of 80 females transplanted between May 1999 and July 2004, 61 were eligible for analysis. Patient characteristics are outlined in Table 2. All patients were transplanted for a haematological malignancy. The duration of surveillance ranged from 6 months to 5 years (median 24 months) with a median number of gynaecological reviews of 7 (range 2–41). Most women attended for regular review and responded positively to genital tract self-surveillance, even those not yet sexually active.

Incidence of genital tract GVHD

Overall, 29/61 women were affected by genital tract GVHD, with a Kaplan–Meier-estimated median time to onset of 2.74 years. The median time to onset was 10 months when only those who developed genital tract GVHD during the observation period were considered. The estimated probability of genital tract GVHD at 1 and 2 years post transplant was 36% (95% confidence interval

(CI) (25–50%)) and 49% (95% CI (36–63%)), respectively (Figure 1).

Clinical aspects

The clinical aspects of patients with genital tract GVHD are detailed in Table 3 and demonstrate that mild, moderate and severe genital tract GVHD occurred in approximately a third each of affected patients. No patient had irreversible vaginal structuring requiring surgery. All patients with genital tract GVHD were on topical oestrogen therapy and all except two patients were on systemic hormone replacement, consisting of oestrogen and progesterone in 15 patients (52%) and oestrogen, progesterone and

Table 2 Patient characteristics

Total patients	61
Median age (years)	42 (19–63)
<i>Graft type</i>	
PBPCs	40
Bone marrow	21
<i>Conditioning</i>	
Non-myeloablative (FluCy)	12
Myeloablative	
• Chemotherapy only (FluMel, BuCy, BuCyVP16)	24
• Chemotherapy and TBI (CyTBI, VP16TBI)	25
<i>Donor</i>	
Sibling	41
Unrelated	20
<i>GVHD prophylaxis</i>	
• With steroids	28
• Without steroids	33

Abbreviations: GVHD = graft-versus-host disease; PBPC = peripheral blood progenitor cell; TBI = total body irradiation. FluCy-fludarabine 125 mg/m² and cyclophosphamide (high) 120 mg/kg or cyclophosphamide (low) 2g/m². FluMel-fludarabine 125 mg/m² and melphalan 120 mg/m². BuCy-busulphan 16 mg/kg and cyclophosphamide 120 mg/kg. BuCyVP16-busulphan 16 mg/kg, cyclophosphamide 120 mg/kg and etoposide 500 mg/m². CyTBI-cyclophosphamide 120 mg/kg and TBI 12 Gy. VP16TBI-etoposide 60 mg/kg and fractionated TBI 1320 cGy.

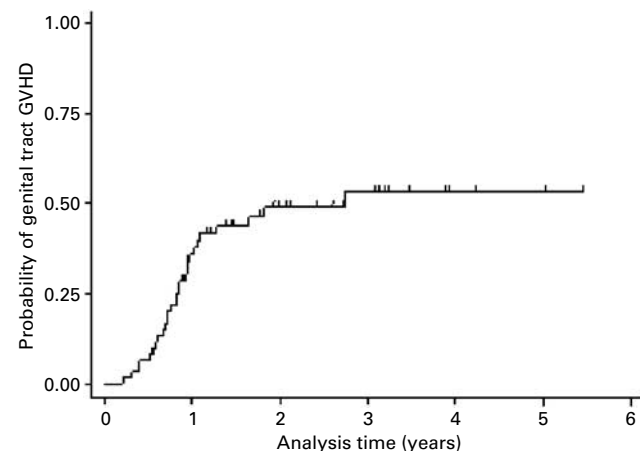


Figure 1 Probability of genital tract GVHD versus time.

androgen in 12 patients (41%). Nearly all patients (90%) had some degree of chronic GVHD in other organs, and 69% experienced prior acute GVHD that was predominantly Grade I–II in severity. The overall incidence of chronic GVHD during the study period was 80%.

Twenty-two of 29 women with genital tract GVHD had resumed sexual activity post transplant, the proportion being similar to those without genital tract GVHD (22/32). At last gynaecological review of the 22 sexually active patients treated for genital tract GVHD, six patients reported persistent dyspareunia (of whom three had also reported pre-transplant dyspareunia) and 14 patients

reported no dyspareunia, with no data available for two patients. Of the sexually active patients who did not develop genital tract GVHD during the follow-up period, only one patient reported dyspareunia at last gynaecological review.

Risk factors and associations

On multivariate analysis, stem cell source was the only variable that conferred an independent risk factor for genital tract GVHD; peripheral blood progenitor cells (PBPCs) were associated with a higher risk than bone marrow-harvested cells (HR = 3.07. (1.22, 7.73), $P = 0.017$) as shown in Figure 2. There was some evidence to suggest that conditioning regimen may influence the incidence of genital tract GVHD, although the results were inconclusive (likelihood ratio test: $\chi^2(2) = 3.72$, $P = 0.155$). Myeloablative regimens appeared likely to confer higher risk (Table 4). There was no evidence that donor source, GVHD prophylaxis, donor sex or age of recipient or donor, influenced the risk of genital tract GVHD independent of stem cell source in this cohort.

Table 3 Characteristics of patients with genital tract GVHD

Total patients	29
<i>Severity genital tract GVHD</i>	
Mild	10
Moderate	10
Severe	9
<i>Prior acute GVHD other organs</i>	
Nil	9
Grade I–II	19
Grade III–IV	1
<i>Chronic GVHD other organs</i>	
Nil	3
Limited	8
Extensive	18
<i>Chronic GVHD organ site</i>	
Oral	23
Skin	18
Liver	11
Gut	5
Other (pulmonary, ocular)	7
<i>Treatment for genital tract GVHD</i>	
Nil	1
Topical steroid	21
Topical cyclosporine and steroid	7
Surgery	0

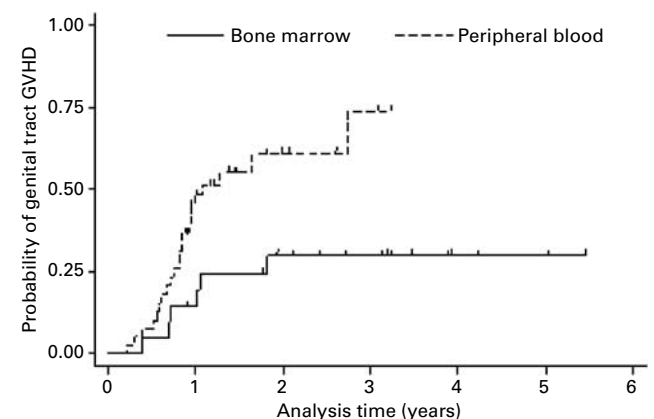


Figure 2 Probability of developing genital tract GVHD according to stem cell source.

Table 4 HRs for prognostic variables for genital tract GVHD

Variable	HR ^a	95% CI	P-value
Peripheral blood versus bone marrow stem cells	3.07	(1.22–7.73)	0.017
<i>Conditioning^b</i>			
Non-myeloablative versus myeloablative chemo regimen	2.54	(0.81–7.95)	0.110
Non-myeloablative versus myeloablative chemo/TBI	2.78	(0.85–9.10)	0.092
Non-sibling donor versus sibling donor	1.01	(0.43–2.35)	0.979
Steroid versus no steroid GVHD prophylaxis	1.51	(0.66–3.45)	0.323
<i>Disease category^c</i>			
Myeloproliferative versus acute leukaemia/MDS	1.12	(0.38–3.31)	0.841
Myeloproliferative versus lymphoma/CLL/myeloma	0.95	(0.28–3.28)	0.936
Age (per 10 years)	1.00	(0.73–1.36)	0.978
Donor age (per 10 years)	1.16	(0.86–1.58)	0.336
Male versus female donor	0.64	(0.30–1.38)	0.258

Abbreviations: CI = confidence interval; CLL = chronic lymphocytic leukaemia; GVHD = graft-versus-host disease; HR = hazard ratio; MDS = myelodysplasia; TBI = total body irradiation.

^aHRs shown are adjusted for the effect of stem cell source.

^bReference category 'non-myeloablative'; HR = 1.

^cReference category 'myeloproliferative disorders' (chronic myeloid leukaemia; essential thrombocytosis); HR = 1.

The observed median time to the onset of non-genital tract chronic GVHD was similar whether the genital tract became involved or not at 6 and 7 months, respectively. Both extensive and limited oral, skin and gut chronic GVHD within the first 12 months post transplant were weakly associated with an increased risk of genital tract GVHD (oral GVHD: OR = 1.93 (0.53, 7.23), $P=0.390$; skin GVHD: OR = 2.36 (0.59, 9.31), $P=0.215$; gut GVHD: OR = 1.94 (0.23, 15.98), $P=0.655$) when 53 patients who had at least 12 months of follow-up were assessed. No association between chronic liver GVHD and genital tract GVHD in the same time interval was found (liver GVHD: OR = 0.85 (0.19, 3.5), $P=1.000$). Sclerodermatous skin changes were more frequent in women affected by genital tract GVHD (50%) than those not affected (27%). Extensive chronic GVHD was more common in patients with genital tract GVHD than those without, occurring in 62 and 28%, respectively.

Treatment and response

The average time to first gynaecological review was 3.5 months (range 1–24 months). A single patient did not receive topical immunosuppression owing to late presentation at 24 months with reduced vaginal capacity and no active inflammatory change. The remaining 28 patients received daily topical steroids with seven patients needing the addition of topical cyclosporine. Dilators were required in nine patients.

Of the 28 treated patients, 15 patients had complete resolution of their symptoms and signs, eight patients showed improvement and five patients had stable disease. The median time to complete response was 12 months with a median treatment duration of 15 months (range 4–37 months) in these patients; four patients were continuing a weaning dose of topical vaginal therapy three times per week. One woman who responded completely and ceased therapy suffered recurrence of genital tract inflammation, which responded to re-treatment. At the time of assessment, all patients with improvement or stabilization of genital tract disease were continuing therapy, with a median duration of treatment of 16.5 months (2–48 months). Although numbers are small, there is a suggestion that complete resolution was more likely in mild genital tract GVHD cases (60%) compared to moderate (50%) and severe (44%) cases.

Figure 3a and b details the histological appearances of the vaginal epithelium in a patient with GVHD before and after the application of topical cyclosporine for steroid-resistant inflammation.

Discussion

Our audit confirms genital tract GVHD to be an important and under-recognized complication of allogeneic SCT with the potential to impair quality of life and sexual function. The main risk factor on multivariate analysis was stem cell source, with PBPC grafts posing a greater risk than marrow, consistent with other reports documenting a higher incidence of chronic GVHD with PBPC.^{9,10} Not

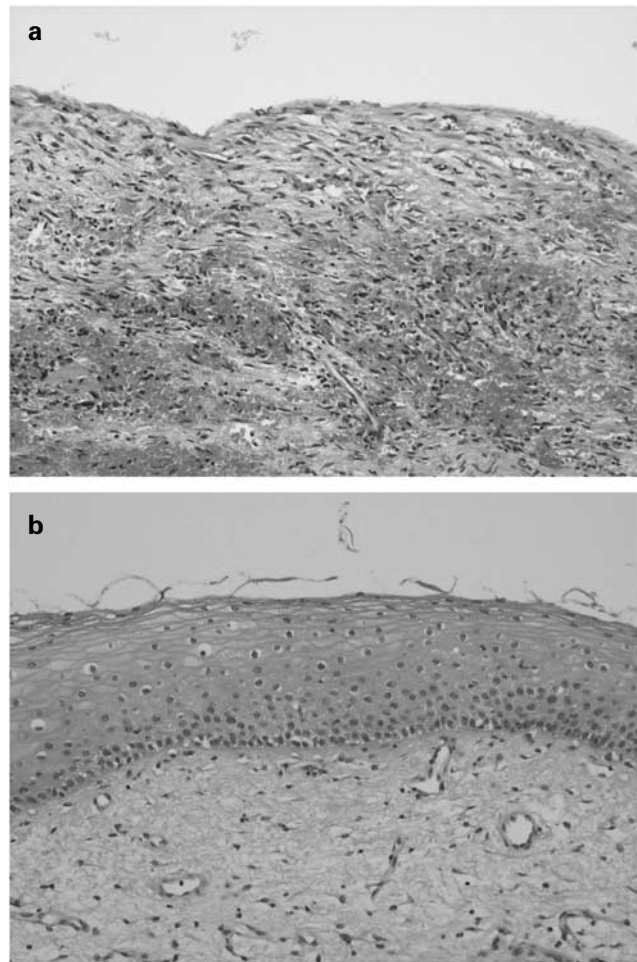


Figure 3 (a) Vaginal biopsy demonstrating ulceration with chronically inflamed, haemorrhagic vascular fibrous tissue focally invested in granulation tissue ($\times 100$), CMV and HSV1/2 immunohistochemistry was negative. (b) Vaginal biopsy demonstrating healing after topical cyclosporine therapy.

surprisingly, genital tract GVHD was almost always associated with chronic GVHD in other organs, particularly the oral mucosa, skin and gut, and was preceded by acute GVHD in over two-thirds of patients. Hence, a high index of suspicion for genital tract GVHD is particularly warranted in women with extensive chronic GVHD. Another important clinical observation is that seven of the 29 cases occurred beyond 12 months post transplant; therefore, the possibility of late-onset disease indicates that long-term gynaecological follow-up is necessary.

The incidence of genital tract GVHD in our series was higher than that reported previously,¹ perhaps reflecting the higher proportion of PBPC grafts in this series and increased detection of mild cases owing to an active surveillance programme.^{9,10} All patients used topical vaginal oestrogen and almost all were also on systemic hormone replacement, eliminating the possibility of atrophic oestrogen deficiency changes mimicking mild genital tract GVHD. We also demonstrated a spectrum of disease severity, suggesting that the predominance of severe

Table 5 Summary of surveillance recommendations for genital tract GVHD

Pre-transplant	Education covering oestrogen deficiency symptoms and symptoms of genital tract GVHD Baseline sexual function assessment
Post transplant: within the first 3 months	Introduction to self surveillance Commence topical vaginal oestrogen Commence systemic oestrogen and progestogen replacement according to age: <40 combined oral contraceptive pill 40–55 HRT > 55 HRT only if oestrogen deficiency symptoms present
Post transplant at 3 months and, in the absence of genital tract GVHD, subsequent 3 monthly gynaecological review until 18 months, then 6 monthly until 3 years and annually thereafter. More frequent assessment as indicated if GVHD.	Review hormone therapy Review sexual function/or vaginal self-surveillance Check for symptoms and signs of genital tract GVHD
Post transplant 6 months	Consider addition of androgen replacement with transdermal 1% testosterone cream or tibolone, if symptoms of androgen deficiency present
Yearly	Cervical cytology

Abbreviations: GVHD = graft-versus-host disease; HRT = hormone replacement therapy.

stenosing lesions in previously published case reports^{2,3} may reflect a reporting bias. An alternative explanation is that our surveillance and early intervention programme altered the natural history of genital tract GVHD and prevented the progression of mild to moderate pathology to severe disease.

Women responded well to an active self-surveillance programme. Topical vaginal immunosuppression, with or without dilator use, resulted in a complete response in over half the patients and prevented disease progression in the remainder, although long-term therapy was often required. Previous authors describe the use of surgical division of adhesions for the management of severe genital tract GVHD,¹¹ which may be unsuccessful owing to scarring and ongoing fibrosis producing recurrent vaginal stenosis. Surgery was completely avoided in our series by the early introduction of serial vaginal dilation in addition to topical therapy. Although over half of the women affected by, and treated for, genital tract GVHD reported no dyspareunia at the end of the audit period, overall these affected females had a higher likelihood of reporting dyspareunia than those unaffected by genital tract GVHD.

A potential active surveillance programme for genital tract GVHD is outlined in Table 5. Essential aspects are pre-transplant counselling to encourage patient participation, early hormone replacement and regular post transplant gynaecological review. The risks and benefits of

hormone replacement therapy should be discussed with each patient at initiation and regularly thereafter. We propose a systematic approach to detection and treatment of genital tract complications post transplant, necessitating close cooperation between bone marrow transplant physicians, transplant co-ordinators and gynaecologists. The incidence and severity of genital GVHD in women should be included as end points in studies of GVHD intervention.

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