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ORIGINAL ARTICLE

Epidemiology and clinical profiles of cutaneous graft versus host disease in allogeneic peripheral blood stem cell transplantation

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Abstract

Introduction: The epidemiology of cutaneous graft versus host disease (GVHD) in allogeneic peripheral blood stem cell transplantation (PBSCT) in Malaysia has not been described. *Materials and Methods:* We retrospectively analysed 691 allogeneic PBSCT patients between 2010-2017 in two centers. *Results:* The prevalence of cutaneous GVHD was 31.4% (217/691). No associations were detected with race, age or gender of donor and recipients. Cutaneous GVHD was associated with host cytomegalovirus (CMV) seropositivity ($p<0.01$), conditioning ($p<0.01$), GVHD prophylaxis ($p=0.046$) and survival ($p<0.01$). Majority developed the acute form (58.1%;126/217). Biopsies in 20.7% (45/217) showed 55.6% positivity for GVHD. Overall, involvement was non-severe. A majority demonstrated complete response (CR) to first-line corticosteroids (70.0%;152/217). Second-line therapies (extracorporeal phototherapy (ECP), psolaren ultraviolet A (PUVA), mycophenolate, tumour necrosis factor (TNF) inhibitors, interleukins inhibitors, or CD20 monoclonal antibodies) were required in 65/217, with 38.5% CR. Second-line therapy was associated with gender ($p=0.042$), extra-cutaneous GVHD ($p=0.021$), treatment outcomes ($p=0.026$) and survival ($p=0.048$). Mortality in cutaneous GVHD was 24.0% with severe sepsis being the leading cause at Day 100 (7.8%) and 5-years (7.8%), and relapsed disease at 2-years (32.7%). In steroid refractoriness, severe GVHD caused 30.8% mortality. In cutaneous GVHD, survival at Day 100 was 95.4%; 80.2% at 2-years and 73.1% at 5-years. The median survival in cutaneous GVHD was significantly shorter at 55 months, compared to those without GVHD at 69 months ($p=0.001$). *Conclusion:* Cutaneous involvement is the commonest clinical manifestation of GVHD. A larger national study is warranted to further analyse severity and outcome of multiorgan GVHD, and factors associated with steroid refractoriness.

Keywords: Allogeneic stem cell transplantation, cutaneous, graft versus host disease (GvHD).

INTRODUCTION

Stem cell transplantation is a standard of care for haematological malignancies, bone marrow failure syndromes, and hereditary haemoglobinopathies. In allogeneic PBSCT, the stem cells are sourced from matched sibling; unrelated or cord blood donor, and haplo-identical donor.

Despite efforts to closely match donor's major HLA classes [Class I (A, B, C) and Class II (DR, DQ, DP)], allogeneic HSCT remains associated with GVHD. The earliest and commonest presentation of acute GVHD is cutaneous, in addition to the gut and liver.[1] The diagnosis is made clinically with high index of suspicion. Naturally, HLA molecules present foreign peptide to T-cells and prevent T-cells from recognising "self" as foreign. In allogeneic PBSCT, the HLA molecules need to

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be overcome and manipulated to allow donor stem cell engraftment. GVHD arises when donor T-cells respond to recipients' HLA and begin an immunological attack against target organs especially the skin, liver, gut and lungs. Disparities in minor HLA classes, and genetic polymorphisms in both donors and recipients of cytokines are thought responsible for GVHD.[2] Other risk factors are recipient and donor older age, gender mismatch, myeloablative conditioning (MAC), donor multiparity, non-conventional GVHD prophylaxis, recipient seropositivity for CMV and the use of PBSC as donor source.[3]

GVHD is classically divided into acute (≤100 days) and late onset or chronic types (>100 days), although it is increasingly recognised that signs of acute and chronic GVHD may occur outside of these periods.[4] Hence, clinical description is more useful to differentiate between the acute from chronic GVHD. Severe acute GVHD occur in 28% of HLA matched unrelated donor and 30% in HLA-matched sibling transplants.[5] The severity is directly correlated with death, where transplant mortality rates (TRM) for grades I-IV acute GVHD was 27%, 43%, 68% and 92%, respectively.[6] Meanwhile, chronic GVHD is significantly associated with negative quality of life and causes major morbidity and mortality in the long-term. However, GVHD is associated with beneficial immunological effect known as graft versus leukaemia (GVL) that prevent relapse and prolong disease remission.

Acute cutaneous GVHD may progress into chronic non-sclerotic or sclerotic GVHD that resemble inflammatory and autoimmune disorders.[7] Hence, the overlapping clinical and histopathological findings can pose a diagnostic challenge. Most of the published data were derived from Caucasian or Oriental patients with naturally fair skin. In contrast, the local darker skin tones may make early clinical diagnosis of erythroderma or erythaematous maculopapular rash challenging.

Classically, acute cutaneous GVHD is described as erythaematous, maculopapular morbilliform eruptions starting on the face, ears, palms, soles and trunk, and may evolve to erythroderma.[8] Follicular erythaema is seen frequently. Epidermolysis can be present in severe cases resembling toxic epidermal necrolysis.[9] Pruritus is associated, but some remain asymptomatic. Atypical presentations e.g. pityriasis rubra pilaris, acquired ichthyosis, and psoriasis vulgaris-like eruption have been reported.[7] These cutaneous descriptions may favor acute GVHD especially if associated with diarrhea and hyperbilirubinaemia.[1] The use of dermoscopy showed that acute cutaneous GVHD may have early clinical presentation with pinkish or reddish background with well-visible, multiple telangiectasias.[10] Chronic cutaneous GVHD is classified as lichen planus-like or sclerotic like scleroderma but now it has been recognised to be a spectrum of epidermal and sclerotic dermal changes.[7]

Skin biopsies is recommended in many guidelines but it must not delay early management in order to improve prognosis. Correlation between clinical and histopathological findings is essential in management.[11] The histopathological grading for acute GVHD may be described as Grade 1 with focal or diffuse vacuolar alteration of basal cells, Grade 2 with vacuolar alteration of basal cells, spongiosis and dyskeratosis of epidermal cells, Grade 3 with formation of subepidermal cleft in association with dyskeratosis and spongiosis, or Grade 4 with complete loss of epidermis.[12] Supportive management e.g. sunscreen and avoidance of sun is combined with definitive treatment i.e. corticosteroids, immunosuppressants, or phototherapy with PUVA or ECP.

This study was conducted in two centres to investigate the prevalence, clinical profiles, severity, treatment outcomes and survival rates. The findings may assist physicians in resource planning and facilitating access to second or third line therapy in severe and refractory cases.

MATERIALS AND METHODS

Study design

A retrospective study was conducted in UKMMC and Hospital Ampang from January 2010 - December 2017 (8 years) involving 691 allogeneic PBSCT patients. Data was extracted from case notes, computer database and national registry and entered to data worksheets. The study was ethically approved by Universiti Kebangsaan Malaysia (UKM JEP-2019-295; UKM FF-2019-223) and Medical Research & Ethics Committee/ MREC Kementerian Kesihatan Malaysia (NMRR-19-44-46608).

Clinical diagnosis of cutaneous GVHD

Acute cutaneous GVHD was clinically diagnosed by a transplant physician based on presence of erythaematous maculopapular rashes, follicular erythaema and epidermolysis resembling toxic

epidermal necrolysis. The severity was graded according to The Modified Seattle Glucksberg Criteria where Grade 0: no rash related to GVHD, bilirubin < 2mg/dL, and no diarrhea; Grade 1: maculopapular rash < 25% BSA without associated symptoms, bilirubin 2 to < 3mg/dL, with nausea, vomiting and diarrhoea > 500-1000 mL/d; Grade 2: maculopapular rash, or erythaema with pruritus, or other associated symptoms covering ≥ 25% to < 50% BSA, or localised desquamation, bilirubin 3 to < 6mg/dL, with nausea, vomiting and diarrhoea > 1000-1500 mL/d; Grade 3: generalised erythroderma, or symptomatic macular/ papular, or vesicular eruption with bullae, or desquamation of ≥ 50% body, bilirubin 6 to < 15mg/dL, with nausea, vomiting and diarrhoea > 1500 mL/d; Grade 4: generalised exfoliative dermatitis, ulcerative dermatitis or bullae, bilirubin ≥ 15mg/dL, with severe abdominal pain with or without ileus.[13] Chronic cutaneous GVHD was clinically diagnosed by a transplant physician upon presence of scleroderma or lichen planus-like. The National Health Institute 2014 (NIH) consensus was used to grade its severity where cutaneous features such as maculopapular rash/ erythaema; lichen-like planus features; sclerotic features; papulosquamous lesions or ichthyosis; or keratosis pilaris-like GVHD were graded into BSA involvement Score 0; Score 1 (1-18% BSA); Score 2 (19-50% BSA) or Score 3 (> 50%).[14]

Histopathological analysis of cutaneous GVHD

Skin biopsy was performed if there were doubt on the aetiology of rash (drug reaction, vasculitis, or viral exanthem) or in patients with no improvement to first line therapy. Under local anesthesia, a punch biopsy was performed as per standard protocol. If present, cutaneous GVHD were graded as Grade 1: focal or diffuse vacuolar alteration of basal cells; Grade 2: vacuolar alteration of basal cells, spongiosis and dyskeratosis of epidermal cells; Grade 3: formation of subepidermal cleft in association with dyskeratosis and spongiosis and Grade 4: complete loss of epidermis.[12,15]

Data collection

Standardised data worksheets were used to collect the following: haematological diagnosis, age and gender for recipients and donors, recipient CMV seropositivity, types of conditioning (MAC; total body irradiation/TBI based; reduced toxicity conditioning/RTC), presence of extra-cutaneous GVHD e.g. gut and/ or liver, stem cell dose, extent of HLA match (matched sibling; mismatched sibling; haploidentical; matched unrelated), GVHD prophylaxis (ciclosporin and methotrexate/CSA+MTX; ciclosporin and mycophenolate mofetil/CSA+MMF, or with anti-thymocyte globulin/ATG), day of engraftment and GVHD treatment first line topical or systemic corticosteroids; second line ECP, PUVA, Anti TNF antibody, IL antibody; third line mesenchymal stem cells infusion. GVHD outcomes were recorded after intervention according to percentages of skin recovery.[16] "**Complete response":** resolution of cutaneous GVHD with no additional treatment, "i**mprovement":** ≥25% improvement of cutaneous GVHD, **"progressive":** ≥25% progress in cutaneous GVHD. Data for overall outcome (alive or death) were also collected.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software (version 25.0, SPSS Inc., Chicago, IL, USA) was used. Continuous and categorical variables were displayed as means ± standard deviation (SD) and percentages, respectively. Simple logistic regression analysis was used for the factors associated with addition of second-line therapy. Univariate analysis was used to compare the outcomes between first-line and second-line therapies. $p \leq 0.05$ was considered statistically significant. The probability of survival was estimated, and the differences between groups were determined after applying Kaplan-Meier statistics.

RESULTS

Patients' characteristics

The study algorithm is illustrated in Figure 1. Cutaneous GVHD was clinically diagnosed in 31.4% (217/691) patients and their characteristics were shown in Table 1. Cutaneous GVHD was reported in 47.5% Malay recipients, Chinese 43.3%, Indian 4.6%, Bumiputra Sabah 4.1% and Others 0.5%. The acute form was diagnosed in 58.1% (126/217), while 24.9% (54/217) developed the chronic form and 17.0% (37/217) manifested both acute and chronic forms. Skin biopsy was performed in 20.7% (45/217) patients, of which 55.6% (25/45) were positive for GVHD features; with 68% (17/25) reported as acute form and 32% (8/25) reported as chronic form. The remaining patients (44.4%; 20/25) were negative for cutaneous GVHD and reported as post-inflammatory related hypo-/hyper-pigmentation,

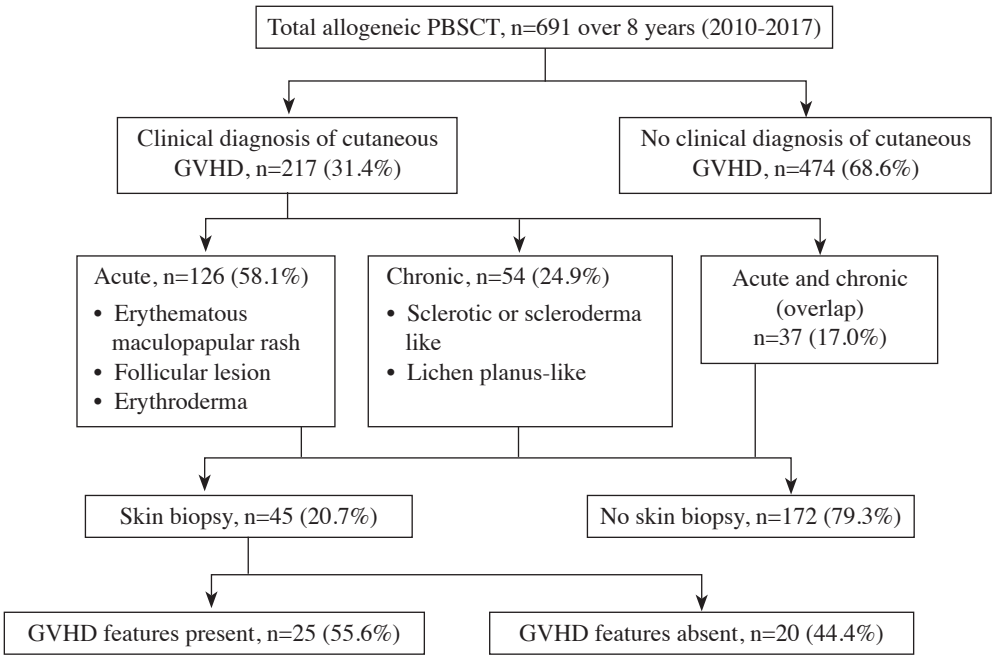

FIG. 1: Study algorithm of cutaneous GVHD in allogeneic PBSCT recipients in HCTM PPUKM and Hospital Ampang between 2010 – 2017.
PBSCT: peripheral blood stem cell transplant; GVHD: graft versus host disease.

drug reactions (lichenoid dermatitis, interface dermatitis) and viral nuclear changes due to herpes infection. Development of cutaneous GVHD was significantly associated with host CMV seropositivity ($p<0.01$), conditioning regimen ($p<0.01$), GVHD prophylaxis ($p=0.046$) and survival ($p<0.01$).

Severity

Acute cutaneous GVHD severity score was shown in Table 2. Overall, the involvement was not severe with Stage 1 28.8% (47/163), Stage 2 53.4% (87/163), Stage 3 13.5% (22/163) and Stage 4 4.3% (7/163). Chronic cutaneous GVHD severity score was shown in Table 3 where Score 1 40.6% (37/91), Score 2 46.2% (42/91) and Score 3 13.2% (12/91).

Outcome

The treatment outcomes of cutaneous GVHD were shown in Figure 2. In addition to optimising therapeutic plasma levels of calcineurin inhibitors, 70% (152/217) received first-line corticosteroids as topical (hydrocortisone or betamethasone) or systemic (prednisolone or methylprednisolone). Here, 49 had cutaneous GVHD alone while 103 had concurrent extra-cutaneous GVHD. The majority achieved CR with first-line therapy (79%, 120/152), 19.1% (29/152) had improved lesions and 1.9% (3/152) had progressive lesions. There were 19.7% (30/152) deaths recorded, including those 3 with progressive lesions who also had concurrent extra-cutaneous GVHD involvement. They died before receiving second line therapy. There were 65 patients (30%) who became steroids refractory and required addition of second-line therapy with ECP, PUVA, MMF, Anti-TNF antibody, or IL antibody after 72-hours of commencement of corticosteroids. Here, 11 had cutaneous GVHD alone while 54 had concurrent extra-cutaneous manifestations (Grade 2-4 GVHD). The majority 53.8% (35/65) showed improved lesions and CR was observed in 38.5% (25/65). Only 5 patients (7.7%) had progressive lesions after second line therapy, who also had concurrent extra-cutaneous GVHD involvement (4 died). Overall, 33.8% (22/65) deaths occurred in patients receiving second line therapy. We detected a significant association between second-line therapy with gender ($p=0.042$), organ involvement of GVHD ($p=0.021$), treatment outcomes ($p=0.026$) and survival ($p=0.048$) (Table 4). GVHD is caused by immune cells dysregulation, compounded by an equally immunosuppressive anti-GVHD therapies. Hence, GVHD and sepsis may occur

Table 1: Characteristics of allogeneic PBSCT patients and clinical diagnosis of cutaneous GVHD

	Total patient, n=691	Cutaneous GvHD, n=217	No GvHD, n=474	p value
Patient age (Mean ±SD)	31.47±13.18	32.14±12.91	31.17±13.30	0.254
Gender, n (%)				0.196
Male	373 (54.0%)	125 (57.6%)	248 (52.3%)	
Female	318 (46.0%)	92 (42.4%)	226 (47.7%)	
Race, n (%)				<0.01
Malay	377 (54.6%)	103 (47.5%)	274 (57.8%)	
Chinese	227 (32.9%)	94 (43.3%)	133 (28.1%)	
Indian	42 (6.1%)	10 (4.6%)	32 (6.8%)	
Sabah	23 (3.3%)	9 (4.1%)	14 (3.0%)	
Sarawak	15 (2.2%)	-	15 (3.2%)	
Others	7 (1.0%)	1 (0.5%)	6 (1.3%)	
Donor age (Mean ±SD)*	31.21±13.82	32.14±13.67	30.81±13.88	0.233
Donor Gender, n (%)*				0.674
Male	403 (59.5%)	124 (58.2%)	279 (60.1%)	
Female	274 (40.5%)	89 (41.8%)	185 (39.9%)	
Host CMV Seropositivity, n (%)*				<0.01
Detected/ Positive	530 (84.9%)	168 (77.4%)	362 (88.9%)	
Not Detected/ Negative	94 (15.1%)	49 (22.6%)	45 (11.1%)	
Diagnosis, n (%)				0.383
Acute Myeloid Leukaemia	309 (44.7%)	93 (42.9%)	216 (45.6%)	
Acute Lymphoblastic Leukaemia	172 (24.9%)	57 (26.3%)	115 (24.3%)	
Chronic Myeloid Leukaemia	61 (8.8%)	25 (11.5%)	36 (7.6%)	
Aplastic Anaemia	56 (8.1%)	12 (5.5%)	44 (9.3%)	
Myelodysplastic Syndrome	34 (4.9%)	12 (5.5%)	22 (4.6%)	
Non-Hodgkin's Lymphoma	20 (2.9%)	6 (2.8%)	14 (3.0%)	
Hodgkin's Lymphoma	16 (2.3%)	5 (2.3%)	11 (2.3%)	
Myelofibrosis	11 (1.6%)	3 (1.4%)	8 (1.7%)	
Haemophagocytic Lymphohistiocytosis	5 (0.7%)	-	5 (1.1%)	
Multiple Myeloma	4 (0.6%)	2 (0.9%)	2 (0.4%)	
Plasma Cell Leukaemia	3 (0.4%)	2 (0.9%)	1 (0.2%)	
Conditioning regimen, n (%)				<0.01
Myeloablative Conditioning	506 (73.2%)	134 (61.8%)	372 (78.5%)	
Reduced Toxicity Conditioning	128 (18.5%)	36 (16.6%)	92 (19.4%)	
Myeloablative + TBI	57 (8.2%)	47 (21.7%)	10 (2.1%)	
HLA matching, n (%)				0.102
Fully matched sibling	600 (86.8%)	179 (82.5%)	421 (88.8%)	
Haploidentical	41 (5.9%)	16 (7.4%)	25 (5.3%)	
Matched unrelated donor	39 (5.6%)	16 (7.4%)	23 (4.9%)	
Mismatched siblings	11 (1.6%)	6 (2.8%)	5 (1.1%)	
GVHD Prophylaxis, n (%)*				0.046
CSA based	674 (97.8%)	216 (99.5%)	458 (97.0%)	
Non CSA based	15 (2.2%)	1 (0.5%)	14 (3.0%)	
Stem Cell Dose ($x10^6$/kg) (Mean ±SD)*	5.41±2.16	5.44±1.99	5.40±2.24	0.952
Outcome, n (%)				0.924
Survival	527 (76.3%)	165 (76.0%)	362 (76.4%)	
Death	164 (23.7%)	52 (24.0%)	112 (23.6%)	
Survival months (Mean ±SD)	55.4±31.16	48.01±31.57	58.79±30.41	<0.01

*There were some missing data. PBSCT: peripheral blood stem cell transplant; GVHD: graft versus host disease; CSA: cyclosporine; HLA: human leukocyte antigen; CMV: cytomegalovirus, TBI: total body irradiation. p-value ≤ 0.05 is statistically significant.

Table 2: Severity assessment for acute cutaneous GVHD [13], n=163.

		Frequency	Percentage (%)
Stage	1: Maculopapular rash <25% BSA	47	28.8
	2: Maculopapular rash >25% <50% BSA	87	53.4
	3: Generalised erythroderma >50% BSA	22	13.5
	4: Generalised exfoliative dermatitis or bullae	7	4.3

GVHD: graft versus host disease, BSA: body surface area.

Table 3: Severity grading for chronic cutaneous GVHD [14], n=91.

		Frequency	Percentage (%)
Score	1: 1-18% BSA	37	40.6
	2: 19-50% BSA	42	46.2
	3: >50% BSA	12	13.2

GVHD: graft versus host disease, BSA: body surface area.

concomitantly with significant morbidity and mortality. The mortality rate in cutaneous GVHD was 24.0% (52/217) with median survival 55 months (Figure 3). Mortality due to severe sepsis with underlying controlled GVHD was 25% (13/52) and this was the leading cause for mortality at 100 days (7.8%) and at 5 years (7.8%). Mortality due to severe GVHD Grade 3-4 with underlying sepsis was 30.8% (16/52), where patients also had concurrent extra-cutaneous GVHD in the lungs, gut or liver. Mortality from disease relapse accounted for 44.2% (23/52) and this was the leading cause for mortality at 2 years (32.7%). Survival rate in cutaneous GVHD at Day 100 was 95.4%; 80.2% at 2 years and 73.1% at 5 years. Survival rate for patients with no GVHD at Day 100 was 95.6%; 88.2% at 2 years and 78.2% at 5 years. The median survival for cutaneous GVHD was 55 months, significantly shorter compared to patients with no GVHD (69 months; p=0.001) (Figure 3). Compared to published data, survival were observed to be higher at 100 days (95.4% vs 85.1%) and 5 years (73.1% vs 44.5%), while

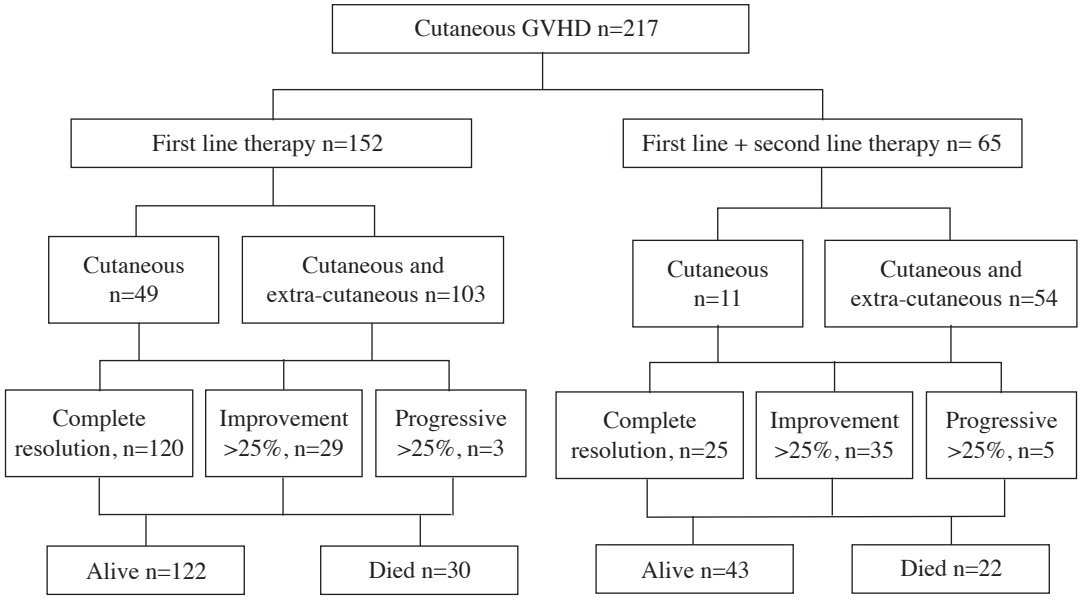

FIG. 2: Treatment outcomes of patients with cutaneous GVHD receiving first line therapy and with addition of second line therapy.

Table 4: Factors associated with lines of treatment for cutaneous GVHD.

	First line, n=152	First line Plus Second Line, n=65	p value
Patient age (Mean ±SD)	32.91±13.51	30.32±11.30	0.147
Gender, n (%)			0.867
Male	87 (57.2%)	38 (58.5%)	
Female	65 (42.8%)	27 (42.4%)	
Race, n (%)			0.442
Malay	67 (44.1%)	36 (55.4%)	
Chinese	71 (46.7%)	23 (35.4%)	
Indian	6 (3.9%)	4 (6.2%)	
Sabah	7 (4.6%)	2 (3.1%)	
Others	1 (0.7%)	-	
Donor age (Mean ±SD)	32.21±14.09	31.96±12.65	0.909
Gender, n (%)			0.042
Male	94 (62.7%)	30 (47.6%)	
Female	56 (37.3%)	33 (52.4%)	
Host CMV Seropositivity, n (%)			0.639
Detected/ Positive	119 (78.3%)	49 (75.4%)	
Not Detected/ Negative	33 (21.7%)	16 (24.6%)	
Diagnosis, n (%)			0.256
Acute Myeloid Leukaemia	72 (47.4%)	21 (32.3%)	
Acute Lymphoblastic Leukaemia	33 (21.7%)	24 (36.9%)	
Chronic Myeloid Leukaemia	18 (11.8%)	7 (10.8%)	
Aplastic Anaemia	7 (4.6%)	5 (7.7%)	
Myelodysplastic Syndrome	9 (5.9%)	3 (4.6%)	
Non-Hodgkin's Lymphoma	5 (3.3%)	1 (1.5%)	
Hodgkin's Lymphoma	4 (2.6%)	1 (1.5%)	
Myelofibrosis	1 (0.7%)	2 (3.1%)	
Multiple Myeloma	1 (0.7%)	1 (1.5%)	
Plasma Cell Leukaemia	2 (1.3%)	-	
GVHD, n (%)			0.021
Cutaneous Only	49 (32.2%)	11 (16.9%)	
Cutaneous +Other Organ GVHD	103 (67.8%)	54 (83.1%)	
Conditioning regimen, n (%)			0.061
Myeloablative Conditioning	91 (59.9%)	43 (66.2%)	
Reduced Toxicity Conditioning	31 (20.4%)	5 (7.7%)	
Myeloablative + TBI	30 (19.7%)	17 (26.2%)	
HLA matching, n (%)			0.642
Fully matched sibling	128 (84.2%)	51 (78.5%)	
Haploidentical	9 (5.9%)	7 (10.8%)	
Matched unrelated donor	11 (7.2%)	5 (7.7%)	
Mismatched siblings	4 (2.6%)	2 (3.1%)	
GVHD Prophylaxis, n (%)			0.253
CSA + MTX	137 (90.1%)	53 (81.5%)	
CSA + MMF	13 (8.6%)	11 (16.9%)	
CSA	1 (0.7%)	1 (1.5%)	
Tacrolimus + MTX	1 (0.7%)	-	
Stem Cell Dose ($x10^6$/kg) (Mean ±SD)	5.49±2.00	5.31±1.97	0.550
Neutrophils >0.5 x 10^9 (Mean ±SD)	14.74±3.15	13.95±2.88	0.089
Platelets >50 x 10^9 (Mean ±SD)	14.17±4.94	13.33±3.79	0.079
Outcome, n (%)			0.026
Survival	122 (80.3%)	43 (66.2%)	
Death	30 (19.7%)	22 (33.8%)	
Survival months (Mean ±SD)	50.78±31.90	41.54±30.04	0.048

*There were some missing data.GVHD: graft versus host disease; HLA: human leukocyte antigen; CMV: cytomegalovirus, TBI: total body irradiation; CSA: cyclosporine; MTX: methotrexate; MMF: mycophenolate mofetil. p-value ≤ 0.05 is statistically significant.

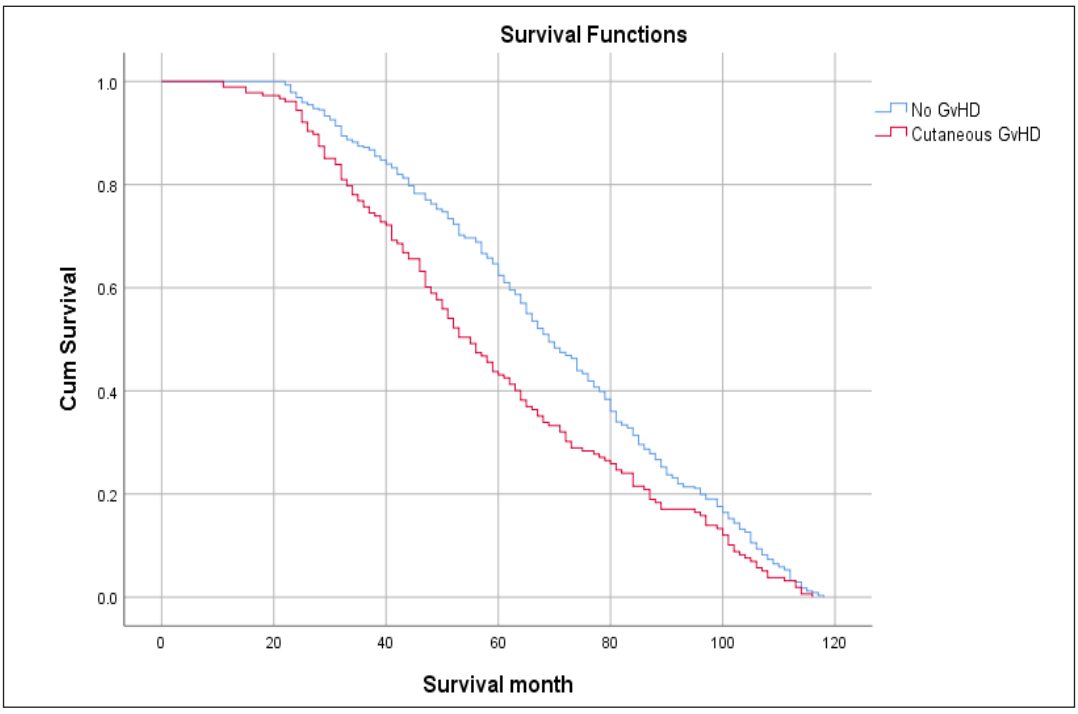

FIG. 3: Median survival for patients with allogeneic PBSCT who developed cutaneous GVHD (55 months) versus without GVHD (69 months), p value=0.001.

mortality was lower (24% vs 51.2%).[17] Tissue biopsies of different stages of cutaneous GVHD are shown in Figures 4A-4B, 5A-5B, 6A-6B, 7A-7B).

DISCUSSION

To the best of our knowledge, this is the first published report on cutaneous GVHD and its clinical profiles amongst allogeneic PBSCT recipients in Malaysia. The prevalence of cutaneous GVHD was 31.4%, slightly lower compared to published data at 40%.[7] Demographically, Bumiputra is the largest ethnic group (67.4%) with Malays being the predominant race (63.1%) followed by Chinese (24.6%), Indians (7.3%) and Others (0.7%).[18] We found no significant association between

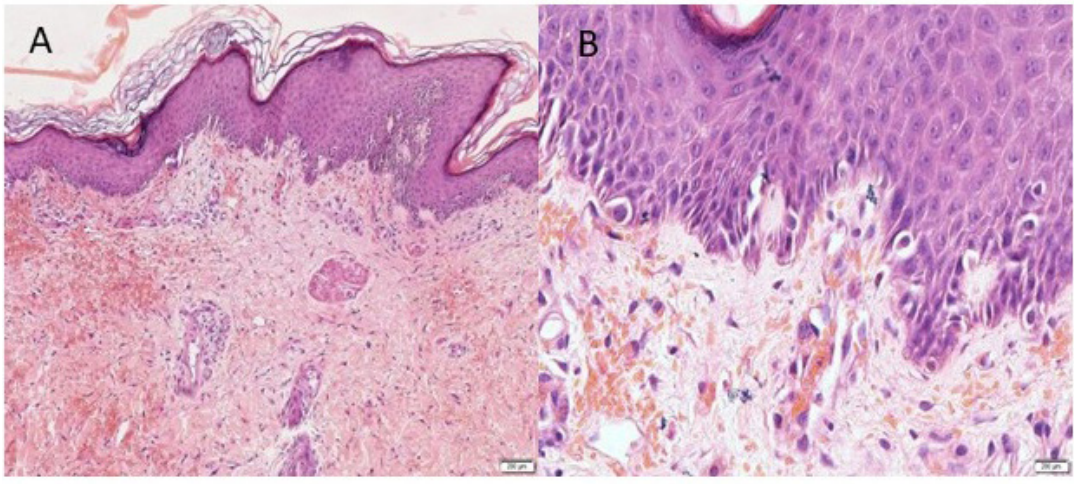

FIG. 4: Acute cutaneous GVHD Grade 1. Normal epidermis appearance with basket weave stratum corneum. No significant lymphocytic cells infiltrate in dermis (4A, H&E, x40). Basal vacuolar change is seen with no dyskeratotic cells (4B, H&E, x400).

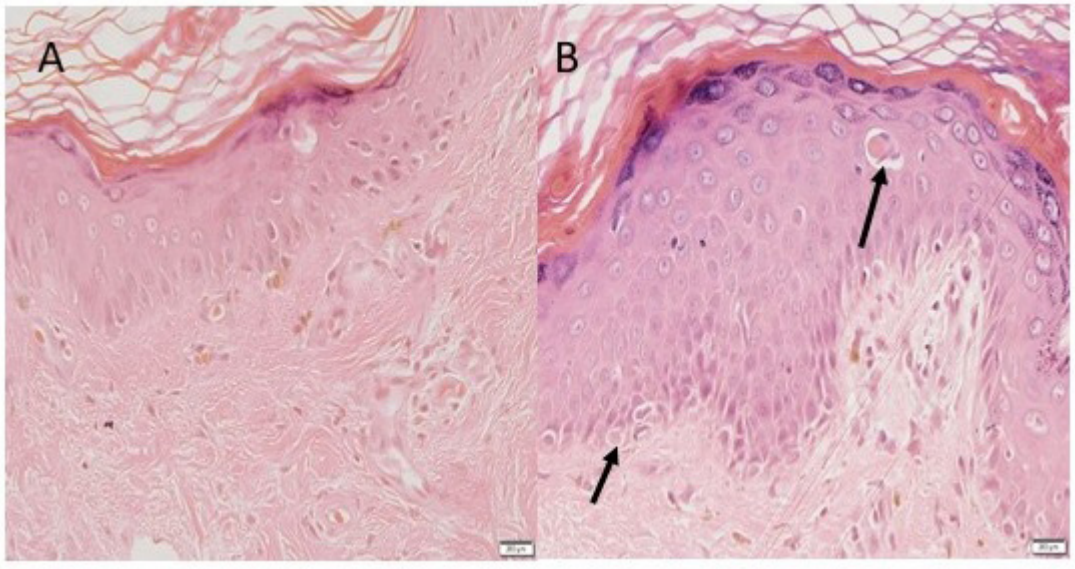

FIG. 5: Acute cutaneous GVHD Grade 2. Prominent melanin pigment incontinence is seen in the papillary dermis (5A, H&E, x200). Basal vacuolar change and dyskeratotic cells are occasionally seen (arrow) (5B, H&E, x400).

race and age or gender of donors and recipients with cutaneous GVHD.

The majority of acute cutaneous GVHD presented with erythaematous maculo-papular rash, while majority of chronic types presented with scleroderma or lichen-like lesions, similar findings to those in published literatures. Cutaneous GVHD was diagnosed clinically, and an invasive skin biopsy was not mandatory unless in clinical uncertainty where other diagnoses needed to be ruled out. In 217 patients with cutaneous GVHD, 20.7% underwent skin biopsy where 55.6% were positive for GVHD. The remaining 44.4% showed alternative diagnosis such as drug reactions, vasculitis, leukaemic infiltration, post-inflammatory hypo/hyperpigmentation, dermatitis and epidermal cysts. Skin biopsy also helped to determine steroid-refractory GVHD and ruled out alternative diagnoses before addition of a more potent and

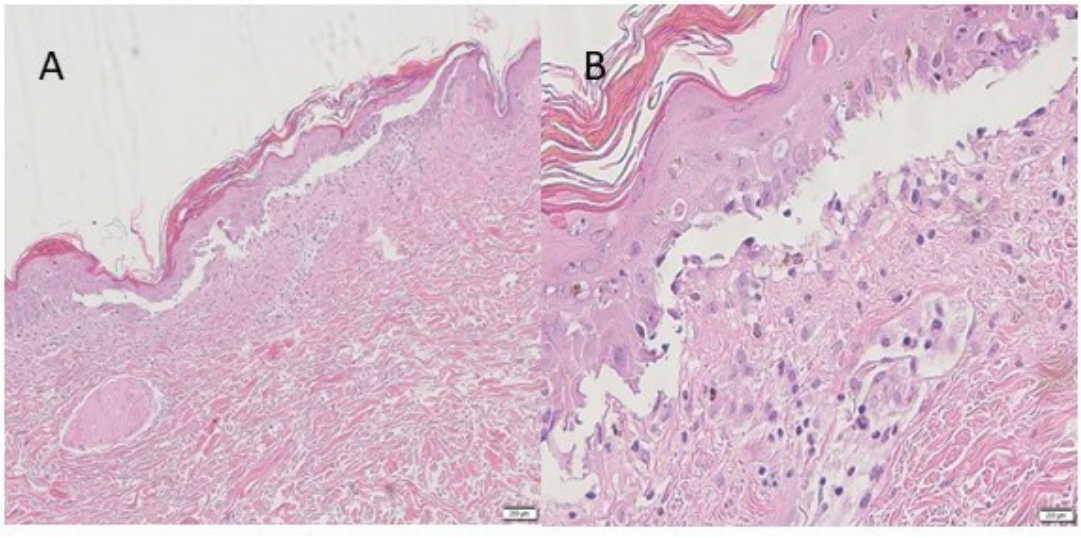

FIG. 6: Acute cutaneous GVHD Grade 3. Prominent subepidermal clefting with overlying parakeratosis (6A, H&E, x40). Subepidermal clefting with dyskeratotic cells at all levels of epidermis with papillary dermal lymphocytic cells infiltrate (6B, H&E, x400).

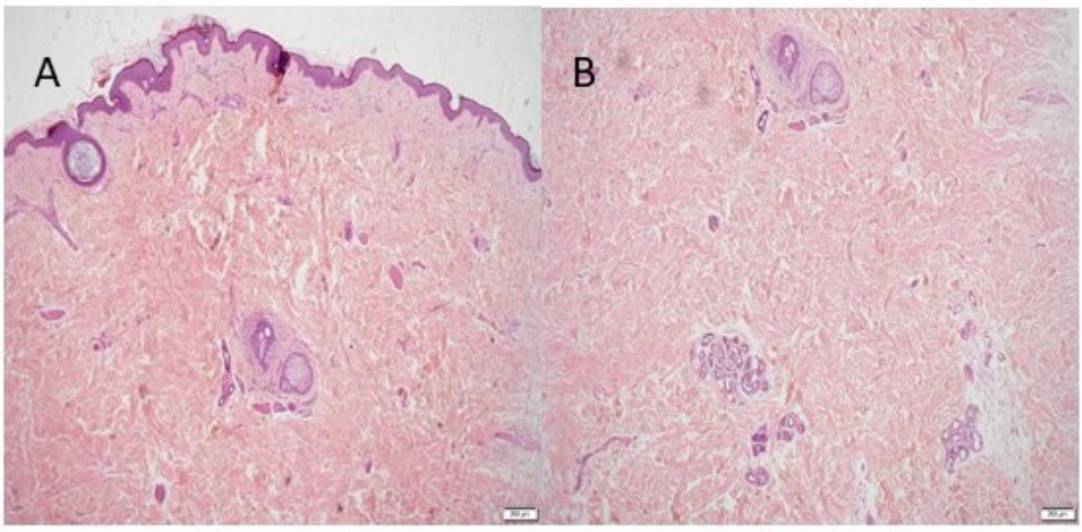

FIG. 7: Chronic cutaneous GVHD. Diffuse dermal sclerosis of skin (7A, H&E, x40). Sclerodermoid dermis with loss of periadnexal fat (7B, H&E, x400).

immunosuppressive second line therapy. In uncertain cases, skin biopsy would change up to 56% of initial diagnosis and affected overall management in 16% of cases.[19] Alternatively, dermoscopy offers a non-invasive bedside approach to aid clinical diagnosis and monitoring of cutaneous GVHD.[20] Vessel granularity and scaling were seen frequently, followed by hyperpigmentation and white patches. The morphology descriptions were moderately correlated with the presence of melanophages and lymphocytic infiltration. Classical sclerotic features were represented as pigmentation and white patches on dermoscopy while lichen planus was represented as pigmentation and granularity. Hence, dermoscopy may be a useful tool to further screen and aid rapid diagnosis of acute cutaneous GvHD in symptomatic patients.[20]

Risk factors for cutaneous GVHD are well described.[17,21] The majority (77.4%) had past infection with CMV (Table 1) which was significantly associated with cutaneous GVHD ($p<0.01$), in agreement with previous reports.[3] GVHD and its immunosuppressive treatments may cause CMV reactivation in patients with pre-transplant CMV serology positivity. Similarly, patients with active CMV replication have a significantly higher risk of developing GVHD compared to patients without CMV replication.[22,23]

The majority received MAC conditioning (61.8%), followed by MAC with TBI (21.7%) and RTC (16.6%) [Table 1]. Conditioning regimen was significantly associated with cutaneous GVHD ($p<0.01$), in agreement with previous reports.[17,21] MAC regimens especially TBI-based inflict extensive tissue damage, which may decrease relapse rates, but caused a higher release of pro-inflammatory cytokines such as TNF-α and IL-1β that are known to be implicated in the development of GVHD.

Almost all patients in our study received CSA based prophylaxis (97.8%), which was significantly associated with development of cutaneous GVHD ($p=0.046$) [Table 1]. However, no significant association was detected between the second line therapy with the use of CSA as prophylaxis (Table 2). In contrast, it was previously noted that the incidence of Grade 2-4 acute GVHD was significantly lower in patients who received tacrolimus based prophylaxis than CSA ($p=0.01$).[24] Similarly, there was a significant trend toward decreased severity of acute GVHD in patients receiving tacrolimus with MTX compared to CSA and MTX.[25] Further analysis is warranted to understand this association further, for example in the monitoring of therapeutic plasma levels of CSA, MTX and MMF in our transplant patients, concomitant presence of gut GVHD that may impair absorption of oral CSA, and liver or renal dysfunctions that may affect drug metabolism.

All recipients with cutaneous GVHD received standard first line therapy topical and systemic corticosteroids with IV Methylprednisolone 1-2 mg/kg/day followed by tapering dose of oral prednisolone. Corticosteroids is established as the standard frontline therapy for acute GVHD.[6,16,26,27] Steroid refractoriness, defined as no clinical response after 5 days or progressively

worsening symptoms after 72 hours[28] were observed in 30% of patients, and was slightly lower than 35-60% reported previously.[7,23] The range may be, in parts due to differences in initiation time of steroid, differing definitions of refractoriness, diverse observation periods, or differences in severity grading.[23] At present, there is no standardised consensus for choice of second line therapy. For most, the determinants were cost, drug availability and safety profiles. Our cohort received therapies aimed for immunomodulation e.g. ECP; PUVA; or inhibition of pro-inflammatory cytokines i.e. TNF inhibitor (Eternercept, Infliximab), Interleukin-2 receptor; or T and B cell suppression with MMF or CD20 monoclonal antibody Rituximab.[29] We observed 38.5% CR and 53.8% improvement to second line therapy, compared to 29.7% for acute GVHD and 44.4% for chronic GVHD as reported previously.[23] The differences may possibly be due to limited study scope to cutaneous alone while others had a larger sample size with GVHD involvement across multiple organ systems. No patients progressed to third line therapy during study period.

In steroid refractoriness, MMF and mTOR inhibitors e.g. Sirolimus showed better response compared to ECP and targeted therapy e.g. Alemtuzumab, Infliximab or Etanercept.[23] There is less data on calcineurin inhibitors as second line therapy due to their extensive usage as prophylaxis and in first line therapy. Steroid refractoriness causes major morbidity and mortality in allogeneic HSCT. Although currently there is no validated prognostic model to identify patients at risk, several parameters e.g. hyperacute GVHD within 14 days of transplant, Grade 3-4 acute GVHD and gender mismatch were identified as predictors to steroid refractoriness.[30] Second line therapy was significantly associated with donor gender (p=0.042). Previously, female donor to male recipients was shown to be associated with an increased risk of acute GVHD and reduced response to first line therapy.[3,23,30,31] Exposure of a parous female donor to non-self-antigens during pregnancy may cause priming of future donor immune system to recognise and attack host antigens. It is unclear why the gender mismatch would also increase the risk for steroid refractory GVHD.[30] A larger sample size and data is required to analyse this association further with regards to donor –recipient gender mismatch and its role in steroid refractoriness.

When limited to the skin, immunomodulation via phototherapy approach were shown to be beneficial for steroid refractory acute GVHD and hence could avoid further systemic immunosuppression agents and its side effects.[7] Grade 2-4 GVHD occurred in 72.4% of our cutaneous GVHD patients as reflected by concurrent extra-cutaneous organ involvement, and this was significantly associated with second line therapy (p=0.021). Severe GVHD Grade ≥2 with gut or liver involvement were known to be significantly associated with a worse response to therapy.[23,30]

We showed that cutaneous GVHD was associated with mean survival (p<0.01) while second line therapy was associated with outcome (p=0.026) and mean survival (p=0.048). The median survival for cutaneous GVHD was significantly shorter at 55 months (p=0.001). In agreement with the previous studies, GVHD and steroid refractoriness was associated with shorter survival and increased TRM.[6,26,30,32]

Our study was limited by missing data due to incomplete records and missed follow ups during the study period. This was due to patients returning to their respective initial referring hospitals once discharged from inpatient transplant admission.

CONCLUSION

Cutaneous GVHD is the commonest and the earliest clinical manifestation of donor T cell alloreactivity. There is a fine balance between a desirable effect of GVL and severe GVHD that causes significant morbidity and mortality. An important factor to consider is an increased risk of cutaneous malignancy from the use of CSA. Direct sun exposure must be avoided, and the use of sunscreen and appropriate clothing must be applied. A larger national study across Malaysia is warranted to further analyse the involvement, severity and outcome of multiorgan GVHD. A standardised online chart for cutaneous GVHD for the sites of involvement, BSA and staging can be made accessible to district hospitals with transplant patients for data registry. More data is required to each treatment modality especially in steroid refractoriness to value different strategies in view of limitations in cost and access to drugs.

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