Functional hyposplenism following allogeneic bone marrow transplantation

R J G Cuthbert, A Iqbal, A Gates, P J Toghill, N H Russell

Abstract

***Aims*—To investigate the incidence of functional hyposplenism in a group of patients who had undergone allogeneic bone marrow transplantation (BMT).**

***Methods*—Splenic function was assessed by counting the number of gluteraldehyde fixed red blood cells containing pits or indentations as examined by interference phase microscopy. Normal values are <2% whereas splenectomy patients have values of 25 to 40%.**

***Results*—Twenty eight BMT recipients (17 men, 11 women) were studied at varying periods post-transplant and the results compared with 20 healthy volunteers and 10 patients who had undergone splenectomy or had splenic atrophy because of haematological conditions. Of the 28 BMT recipients, one had undergone a prior splenectomy; of the remaining 27 patients, four (15%) had evidence of functional hyposplenism with between 5·0 and 34·0% pitted cells. Of these four patients, one had active extensive chronic graft versus host disease (GvHD) which has been previously reported to be associated with functional hyposplenism following transplantation. Only one of the four patients had peripheral blood red cell changes typical of hyposplenism.**

***Conclusion*—These results confirm that extensive chronic GvHD is associated with hyposplenism. Intermediate degrees of functional hyposplenism may also occur following BMT in the absence of chronic GvHD and in the absence of haematological features of hyposplenism on routine blood films. This may be of significance in mediating the susceptibility to infection with encapsulating bacteria seen following allogeneic BMT.**

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Infection with encapsulated bacteria, particularly pneumococci, is a well recognised late complication following allogeneic bone marrow transplation (BMT).[1 2] The risk of serious infection is greatest in patients who develop chronic graft versus host disease (GvHD).[3] Immunoglobulin deficiency, especially IgG subclasses 2 and 4, may be associated with an impaired immune response to pneumococci and other encapsulated bacteria.[1 4] Such immunoglobulin deficiency occurs most frequently in patients with chronic GvHD.[5] Kalhs *et al* have reported the occurrence of functional asplenia in patients with chronic GvHD, which may also contribute to the increased susceptibility to bacterial infections seen in these patients.[6 7]

Functional hyposplenism has been reported in a number of patient groups including those with sickle cell disease, coeliac disease, inflammatory bowel disease, and amyloidosis. These patients, and also those who have undergone splenectomy, are known to have an increased susceptibility to infections with encapsulated bacteria.

Severe functional hyposplenism, splenic atrophy and splenectomy are associated with the presence of characteristic morphological changes in red blood cells, including Howell–Jolly bodies, acanthocytes, crenated cells, target cells, and spherocytes. With partial splenic dysfunction, these characteristic changes may not be observed and lesser degrees of hyposplenism may only be detected using indium labelled scintiscanning.

Corazza *et al*[8] have described a simple differential interference contrast microscopic technique in which red cell indentations or pits, normally removed by the spleen, can be detected. These pits contain vacuoles with ferritin, haemoglobin and mitochrondrial remnants.[9] The technique has been shown to be significantly more sensitive than detection of Howell–Jolly bodies on blood films, and correlates well with the degree of hyposplenism as assessed by scintiscans.[8] We have applied this technique to investigate the prevalence of functional hyposplenism in long term survivors following allogeneic BMT.

Methods

The study group consisted of patients who had survived at least six months following allogeneic BMT. As many long term survivors become infrequent attenders at follow up clinics, the study group does not consist of consecutively transplanted patients. The study design was a cross-sectional survey based on single samples taken at routine follow up. Results were compared with a group of 20 healthy volunteers (median age 33 years, range 21–50 years) and 10 patients who had undergone splenectomy or had established hyposplenism (median age 33 years, range 10–75 years). In the latter group four patients had undergone splenectomy for immune thrombocytopenia and two for lymphoma or leukaemia, and four patients had splenic atrophy caused by sickle cell disease.

Clinical details and percentage pitted red cell counts in 28 BMT recipients

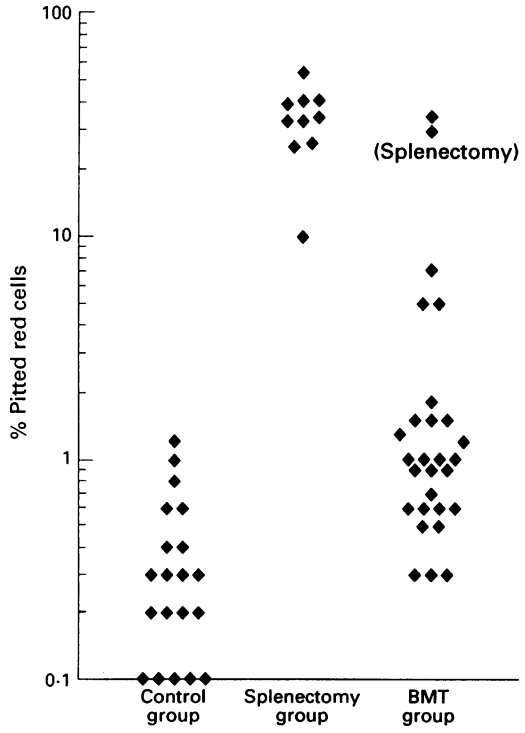
Case No.	Age/sex	Diagnosis	Months post-BMT	Acute GvHD	Chronic GvHD	Red cell pits (%)	Comments
1	41/F	AML	84	–		0·6	
2	23/M	ALL	66	–	Extensive	0·7	Chronic GvHD resolved
3	37/F	AML	61	–		1·5	
4	21/M	AML	58	–		0·3	
5	40/M	MDS	53	–	Limited	29	Splenectomy
6	46/M	MM	37	I		0·9	
7	47/M	AML	35	–		1·2	
8	42/M	AML	34	I		1·0	
9	17/M	AML	33	–		5·0	
10	25/M	AML	32	–		1·0	
11	31/F	AML	32	–		0·6	
12	41/M	CML	28	–		0·3	
13	44/F	MM	25	–		0·9	
14	29/F	CML	24	I		1·5	
15	48/F	MDS	22	–	Extensive	34	
16	52/F	MM	21	–		7·0	
17	26/M	AML	19	–		0·6	
18	42/M	AML	18	I		0·5	
19	15/M	ALL	16	–		1·3	
20	36/M	CML	16	–		1·0	
21	40/M	MDS	15	–		0·6	
22	49/F	MM	13	I		1·8	
23	22/M	SAA	13	I		0·9	
24	29/M	CML	10	–		1·3	
25	45/M	ALL	9	–	Limited	5·0	
26	50/M	MM	8	–		0·5	
27	40/M	CML	8	–	Limited	1·0	BOOP
28	43/F	AML	7	–		0·3	

AML, acute myelobastic leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; MM, multiple myeloma; SAA, severe aplastic anaemia; BOOP, bronchiolitis obliterans with organising pneumonia.

All patients underwent BMT for haematological malignancies or severe aplastic anaemia. The conditioning regimen consisted of cyclophosphamide 120 mg/kg and fractionated total body irradiation (12 Gy). Cyclosporin and methotrexate were administered for GvHD prophylaxis according to our previously published regimen.[10]

Blood films stained using a Romanovsky technique were reviewed for the presence of Howell–Jolly bodies and the other characteristics of functional asplenia. Splenic function was further studied by counting the number of red cell pits according to the method of Corazza *et al.*[8] A wet preparation of one drop of peripheral blood mixed with 0·5 ml 3% gluteraldahyde buffered to pH 7·4 was examined by interference phase contrast microscopy. One thousand red cells were examined to determine the percentage with such pits (normal <2%). All samples were processed and examined by a single observer (AG).

The Mann–Whitney U test was used to compare results between the patient group and the control group.



Distribution of percentage of red cells with pits in 28 allogeneic BMT recipients compared with a group of normal controls (n=20) and a group of patients who had undergone splenectomy or had splenic atrophy caused by sickle cell disease (n=10).

Results

The patient group comprised 28 allogeneic BMT recipients (17 women and 11 men). The median age at BMT was 40 years (range 15–52 years). Median follow up was 25 months (range 10–85 months). The clinical details including diagnosis at BMT and incidence of acute and chronic GvHD are outlined in the table. Six patients developed grade I acute GvHD. Active extensive chronic GvHD was present in one patient (case 15) at the time of the study. One patient (case 2) had extensive chronic GvHD which was in clinical remission. Two patients had limited stage chronic GvHD (cases 25 and 27); case 27 subsequently developed bronchiolitis obliterans with organising pneumonia.

Although recurrent minor bacterial infections occurred in three patients, none had serious bacterial infection 100 days post-BMT. However, one patient at our centre died of pneumococcal septicaemia one year post-BMT, before the current study was undertaken.

The figure shows the distribution of pitted red cell counts in the study group compared with healthy volunteers and patients who had undergone a splenectomy. Twenty three BMT recipients had pitted red cell counts of <2% whereas five patients had results above normal. One patient from this group had previously had a splenectomy for staging of Hodgkin's

disease (case 5) and was excluded from further analysis. Thus, four of 27 (15%) BMT recipients had evidence of functional hyposplenism. As a group, the BMT recipients had significantly higher pitted red cell counts than the normal controls ($U = 4{\cdot}34$; $p < 0{\cdot}001$). During the study, only two BMT recipients had Howell–Jolly bodies detectable on peripheral blood films (case 5 (previous splenectomy) and case 15 (active extensive chronic GvHD)).

Discussion

The prevalence of both acute and chronic GvHD was low in the study group, which is consistent with our previously published experience.[10] Functional asplenia was observed in the one patient who had active extensive chronic GvHD, consistent with the report by Kahls *et al.*[6] However, the technique used for assessing splenic hypofunction by measuring pitted red cell counts is more sensitive than detection of Howell–Jolly bodies on peripheral blood films. Thus, we were able to identify patients with normal blood films who had intermediate degrees of functional hyposplenism and who did not have active extensive chronic GvHD.

As this technique is very simple to perform, regular follow up samples can easily be organised. Therefore, prospective evaluation of splenic function in BMT recipients could be conducted. This data may be of value in determining which patients should be offered long term antibiotic prophylaxis against encapsulated bacteria. We are currently undertaking such a prospective study of the significance of splenic dysfunction in mediating the susceptibility to bacterial infection following BMT.

Our current policy is to recommend penicillin V prophylaxis to all patients for a minimum of two years following BMT. At one year, we recommend polyvalent pneumococcal and *H influenzae* type b vaccination. Patients who develop extensive GvHD are maintained on penicillin V prophylaxis, as antibody responses to vaccination are impaired. Assessment of splenic function at regular intervals could be of additional value in determining patients without chronic GvHD who may benefit from penicillin V prophylaxis for more than two years.

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