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Burst-Forming Unit-Erythroid Assays (BFU-E) To Distinguish Cellular Bone Marrow Failure Disorders

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Abstract

Patients with cytopenias and a cellular bone marrow can be a diagnostic and a therapeutic challenge. Previous reports have suggested a role for progenitor assays as a potentially useful test for diagnosis and predicting response to therapy. Here we report the results of BFU-E assays in 48 consultative cases of single or multi-lineage cytopenias with cellular marrows. The final diagnoses included 17 patients with myelodysplastic syndrome (MDS); 9 patients with pure red cell aplasia (PRCA) [non-large granular lymphocytosis (LGL) in etiology]; 15 patients with LGL (8 of which had a single lineage cytopenia only while the other 7 had multi-lineage cytopenias); and 7 patients with cytopenias associated with systemic inflammation related to autoimmune conditions. In this cohort, nonmalignant diseases were well-distinguished from MDS by BFU-E growth. Our data suggest that low BFU-E growth (less than 10 BFU-E /10⁵ marrow mononuclear cells) helps to exclude LGL, PRCA, or cytopenias associated with systemic inflammation as a cause of pancytopenia with a sensitivity of 96.8%, specificity of 76.5% and a predictive value of 88.2% (p=0.0001). BFU-E growth was also examined to predict response to treatment. Of the 29 patients in this cohort treated with immunosuppressive therapy across all disease groups, there was an 86% response rate with 25 responders (11 PRs and 14 CRs) and 4 non-responders. This did correlate with higher BFU-E growth. Our results suggest BFU-E assays are a useful adjunct in the diagnosis and management of cytopenias in the setting of a normocellular or hypercellular bone marrows.

Keywords

BFU-E; bone marrow failure; pancytopenia

Refractory anemias, bi-lineage, and tri-lineage cytopenias in the context of normocellular or hypercellular bone marrows often present difficult diagnostic dilemmas. The diagnosis can remain unclear even after repeated bone marrow evaluations. Many of these patients are given a diagnosis of possible or probable myelodysplastic syndrome (MDS), even though such a diagnosis can be imprecise in the absence of increased blasts or karyotype

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Conflict of interest

None.

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abnormalities (Tefferi and Vardiman 2009); in fact, discrepancies in the diagnosis of MDS have been reported to be as high as 12% (Naqvi, Jabbour et al. 2011). Importantly, even when only suspected, a diagnosis of MDS can have significant implications therapeutically and emotionally for the patient.

The differential diagnosis in patients with cytopenias and cellular/hypercellular marrows, but without increased blasts or cytogenetic abnormalities, is broad. Once nutritional deficiencies, infections such as human immunodeficiency virus (HIV), hemolytic processes, and drug causes as well as infiltrative diseases of the bone marrow (e.g. leukemias, lymphoproliferative disorders and other malignancies) have been excluded, the differential includes pure red cell aplasia (PRCA), large granular lymphocytosis (LGL), marrow suppression associated with systemic inflammation, immune mediated cytopenias, clonal bone marrow failure disorders such as myelodysplastic syndrome (MDS) and paroxysmal nocturnal hemoglobinuria (PNH). Accurate diagnosis is crucial for correct treatment, and it can be difficult to differentiate between MDS, PRCA, LGL, and inflammatory/ autoimmune disease (Leguit and van den Tweel 2010). Newer molecular and genomic tests such as single nucleotide polymorphism (SNP) arrays and even exon or whole genome sequencing are increasingly being incorporated into these work-ups, but these tests may also not provide a definitive diagnosis particularly for non-clonal processes.

Burst-forming unit - erythroid (BFU-E) and other hematopoietic progenitor assays have previously been shown to predict response to immunosuppressive therapy (IST) for PRCA (Charles, Sabo et al. 1996). Although not routinely available at all centers, these progenitor cell assays can provide a sensitive screening parameter for undiagnosed cytopenias (Tennant, Jacobs et al. 1986; Valent 2012). In MDS specifically, they may also have prognostic significance in that low growth suggests a poorer prognosis (Geissler, Hinterberger et al. 1988; Shih, Chiu et al. 1991). Since 2007 we have been performing these BFUE assays in diagnostically challenging (often 2nd opinion) cases with the hopes of gaining additional insight into better diagnosis of bone marrow failure states. Here, we tested the hypothesis that BFU-E assays are a useful adjunct in the diagnosis of cytopenias in the setting of normocellular or hypercellular bone marrows.

Material and Methods

Human subjects

As part of an IRB-approved study, we performed a retrospective analysis on patients presenting for evaluation of cytopenias from January 2007 through October 2012. Inclusion criteria included a recorded BFU-E assay performed prior to therapy, follow-up for at least 6 months after treatment or until death, and a presumed pathologic diagnosis for the cytopenias further confirmed by the clinical course. BFU-E and other progenitor colony assays have been routinely performed on patients undergoing bone marrow examinations for cytopenias since 2007. The cytopenias were defined as less than the identified normal ranges for age and sex. Anemia was a hemoglobin (Hb) < 13.5 g/dL (male) or 12 g/dL (female), leukopenia was a total white blood cell count (WBC) < $4.0 \times 10^9/L$ or absolute neutrophil count (ANC) < $1.0 \times 10^9/L$ and thrombocytopenia was a platelet (Plt) count < $150 \times 10^9/L$.

The patients' diagnostic testing included peripheral counts, cellularity, antinuclear antibodies (ANA), cytogenetics, (fluorescent *in situ* hybridization (FISH), T cell receptor (TCR) gene rearrangement by polymerase chain reaction, and flow cytometry. Most patients (excluding the systemic inflammation patients) had iron stains, quantitative CD34 counts (Matsui, Brodsky et al. 2006), and flow cytometry for PNH (Brodsky 2009; Borowitz, Craig et al. 2010).

Excess material from healthy donors undergoing harvest allogeneic bone marrow transplant harvests served as controls. Normal donors were required to have normal peripheral blood counts for age and sex, as well as bone marrow cell (MMNC) count $20 \times 10^6/L$, as well as supply informed consent on an IRB-approved specimen collection trial.

Disease definitions

LGL was defined as a uniform population of greater than $0.5 \times 10^9/L$ lymphocytes that expressed CD3, dim CD5, CD8 and CD57 and a positive clonal T cell gene rearrangement identified by polymerase chain reaction with primers specific for the T cell receptor (Lamy and Loughran 2011), or expressed CD8, CD2, CD7, CD16, bright CD45 and CD11c for NK LGL. PRCA was defined as isolated anemia (untransfused Hb $< 8g/dL$) with reticulocytopenia ($< 1\%$) and increased proerythroblasts in the marrow aspirate (Dessypris 1991). MDS was defined as dysplasia with blasts, as per World Health Organization (WHO) criteria (Swerdlow, Campo et al. 2008; Tefferi and Vardiman 2009) or elevated quantitative CD34 count (Matsui, Brodsky et al. 2006). Systemic inflammatory diseases were defined by the rheumatologist who referred them or by positive antibodies in the respective diseases including anti-nuclear antibodies (Cines and Bussel 2005; Michel 2011).

Complete response (CR) was defined as a normal complete blood count for age and sex. Partial response (PR) was defined as improvement in counts and transfusion independence without count normalization. Non-responders (NR) were those patients who did not have improvement in their cytopenias after therapy. Disease-specific response criteria were used for each systemic inflammatory disease. Patients with systemic lupus erythematosus (SLE) met 4 or more of the revised American College of Rheumatology classification criteria for SLE with moderate-to-severe activity.

BFU-E assay

The BFU-E assays were performed in the Clinical Laboratory Improvement Amendments (CLIA) certified Cell Therapy Lab of the Sidney Kimmel Comprehensive Cancer Center of the Johns Hopkins Hospital according to a standard operating procedure. Briefly, each procedure was performed on a single bone marrow aspirate obtained in a lithium heparin tube. Samples were placed in hematopoietic cell culture media with 1 mL α -medium containing final concentrations of 1.2% methylcellulose, 1% bovine serum albumin, 30% fetal calf serum with 1 U/mL recombinant human erythropoietin and 2.5% human lymphocyte conditioned medium. Cells were plated at 1×10^5 cells/mL into three 35×10 mm suspension culture plates and placed into an incubator maintained at 37 degrees Celsius with 5% CO₂ and $>85\%$ humidity. The BFU-E colonies were enumerated after 12–14 days incubation via microscopy. The culture dishes were scored for BFU-Es under an inverted phase contrast microscope and reported as an average of the 3 dishes in BFU-E per 1×10^5 nucleated cells. Colony identification was performed with greater than 50 cells identified as a BFU-E (Pu, Hu et al. 2012). As this was performed as a standardized assay in a CLIA certified lab, only enumeration of the colonies was performed. Size and shape of the colonies was not reported by disease type as these are not standardized parameters. Patient specimens that lacked CD71^{bright} CD45^{negative} nucleated red cells and normal B-cell precursors by flow cytometry (Weir, Cowan et al. 1999) were considered specimens not fit for BFU-E evaluation. This was necessary to demonstrate that CD34 counts and hematogones were present to ensure the sample is adequate to avoid marrow specimens diluted with peripheral that would give misleading results.

Statistics

Statistical analysis included Wilcoxon rank-sum test, Fisher's exact test and the Kruskal-Wallis for comparisons performed with STATAIC 10 (College Station, TX.)

Results

Forty-eight consecutive patients meeting the above criteria for cytopenias with a cellular or hypercellular bone marrow had BFU-E assays and were included in the study. The majority (86%) of the patients had previous outside bone marrow evaluations for their cytopenias, which yielded diagnoses of possible MDS or cytopenias of uncertain etiology. Fourteen of 15 (93%) of the LGL patients had had previous bone marrow biopsies as had 8/9 (89%) of the PRCA patients (89%), 14/17 (82%) of the MDS patients and 3 of 7 (43%) patients with systemic inflammation. All were referred for MDS or to rule out a primary marrow disorder as a cause of systemic illness in the patient.

Tables 1–4 describes patients' baseline data at the time the diagnostic bone marrows with BFU-E assays were performed. The median bone marrow cellularity across all groups was 60 (range, 20–100) percent. The presumed final diagnoses based on pathology and clinical follow-up included 17 patients with MDS; 9 patients with PRCA (non-LGL in etiology); 15 patients with LGL [8 of which had a single lineage cytopenias (7 PRCA and 1 isolated neutropenia) while the other 7 had multi-lineage cytopenias]; and 7 patients with cytopenias from systemic inflammation. The median follow-up was 12 (range, 1–61) months. There were 10 deaths with causes listed in Table 5. There were 24 normal controls for comparison of BFU-E growth. The median growth in the normal controls was 41 BFU-E /10⁵ MMNC (range 24–87).

The 17 patients with MDS had the lowest median BFU-E growth at 5 (range 0–38) /10⁵ MMNC. The majority of these patients had International prognostic scoring system (IPSS) scores (Greenberg, Cox et al. 1997) of intermediate 1 (Int-1). Seven of these patients had diagnoses that were unclear at the time of their marrow evaluation. The remaining 10 had excess blasts or complex karyotypes on the marrow examinations performed at our institution. In this group, 12 patients receive therapy directed against their MDS and the remaining 5 received supportive care alone. Six died from progression to acute leukemia in less than 9 months. One patient underwent allogeneic bone marrow transplantation for her MDS, and another with 5q minus (in a complex karyotype) has responded to lenalidomide. The other patients are alive but remain red blood cell transfusion-dependent with clinical evidence of progression.

There were 9 patients with PRCA (not associated with LGL). Their marrow grew a median of 23 (range 4–53) BFU-E /10⁵ MMNC. Seven of these patients were treated with IST, of whom 5 responded with 2 CRs and 3 PRs. The etiologies of the PRCA included 2 associated with thymoma (one received chemotherapeutic treatment of metastatic thymoma to no evidence of disease and one had no response to IST, though compliance was limited), two with anti-erythropoietin antibodies (both died at 1 [no treatment given] and 11 months), two with ABO-incompatible bone marrow transplants (both achieved a PR with IST), and 3 idiopathic PRCA patients (two achieved CRs with IST).

The 15 patients with LGL grew a median of 69 (range 20–133) BFU-E /10⁵ MMNC. Of these 15 patients, eight exhibited single lineage cytopenias (7 - PRCA, 1 - isolated neutropenia) and grew a median 59 (range 20–133) BFU-E /10⁵ MMNC. The six LGL patients with multi-lineage cytopenias had BFU-E growth of 82 (range 25–105)/10⁵ MMNC (p=0.24). Thirteen patients responded to first or second line IST with 8 durable CRs and 5 PRs of 6 months or greater. One of the non-responding LGL patients with PRCA died at 31 months from infection after multiple relapses and treatments, including an allogeneic transplant six months before death. The median BFU-E growth for the responders was 81 (range 20–133)/10⁵ MMNC compared to 61 (range 21–76) /10⁵ MMNC in the non-

responders ($p=0.31$). One LGL patient later progressed to MDS with increased blasts after a 6 month response with transfusion independence after oral cyclophosphamide.

The 7 patients with cytopenias from systemic inflammation all had documented autoimmunity (1 with Evan's syndrome, 1 with vasculitis, 1 with relapsing polychondritis and 4 with systemic lupus erythematosus). Their marrows generated a median of 104 (range 49–136) BFU-E / 10^5 MMNC. All 7 (100%) patients responded to IST directed at their underlying disease with a hematologic CR in 4 and PR in 3 patients.

The medians and distributions for BFU-E growth from the four diagnostic categories of cytopenic patients with cellular marrows are statistically significantly different ($p=0.0001$, Figure 1) from each other as well as the normal controls. Of note, only 3 of the 17 MDS patients BFU-E growth overlapped any of the normal or LGL patients, and there was no overlap with the patients with inflammatory cytopenias. Moreover, none of the MDS patients' BFU-E numbers overlapped the median BFU-E growth for these three groups. There were 34 patients in this cohort with growth of greater than 10 BFU-E / 10^5 MMNC, and only 4 of them had MDS. Fourteen patients had growth less than 10 BFU-E / 10^5 MMNC and 13 of them had MDS. Thus, growing < 10 BFU-E / 10^5 MMNC essentially ruled out LGL, PRCA, or inflammation as a cause for cytopenias with a sensitivity of 96.8%, specificity of 76.5% and a predictive value of 88.2% ($p=0.0001$).

BFU-E growth was also examined as a predictor of response to treatment in the disease categories for which IST is used as standard of care: PRCA, LGL, and systemic inflammation. In this cohort, there were 29 patients treated with IST across these disease groups. For this group, there was an 86% response rate with 25 responders (11 PRs and 14 CRs) and 4 non-responders. The median BFU-E growth for the non-responders was 37 (range 19–61) / 10^5 MMNC while the median BFUE for the responders was 63.7 (range 10–137) ($p=0.001$). Two patients treated with IST grew less than 30 BFU-E, and neither responded. When looking specifically at growth greater than 30 BFU-E / 10^5 MMNC, there were 18 responders and 2 non-responders with a positive predictive value of 90% ($p=0.38$).

Discussion

The diagnosis of cytopenias in the setting of a cellular bone marrow may be challenging, especially when there is no gross tumor, non-diagnostic morphology, and a normal karyotype. None of the routine assays (morphology, FISH, cytogenetics, iron stains, flow cytometry etc.) provide an assessment of bone marrow growth and differentiation. In adults, the differential diagnosis of cytopenias with cellular bone marrows frequently includes MDS, LGL, red cell aplasia, and systemic autoimmune diseases. Further, many MDS patients have a normal karyotype, and subtle dysplasia is common in LGL and inflammatory disorders.

Here, we demonstrate that BFU-E growth may be an important adjunct to the work-up of previous undiagnosed cytopenias. Importantly, BFU-E assays are relatively inexpensive and provide new data that was not obtained on the original non-diagnostic bone marrow specimen. Our data suggest that low BFU-E growth (less than 10 BFU-E / 10^5 MMNC) is more consistent with a diagnosis of MDS and helps to exclude the diagnosis of red cell aplasia, LGL, or cytopenias from systemic autoimmune diseases. More robust BFU-E growth (> 20 BFU-E per 10^5 cells) is usually associated with external suppression hematopoiesis such as occurs in LGL, red cell aplasia, or autoimmunity. Interestingly, BFU-E growth for patients with LGL associated PRCA was higher than that of patients with non-LGL associated PRCA, but both subtypes had little overlap when compared to BFU-E growth in MDS (Figure 1 and Tables 2 and 3). Although we had only four patients treated

with IST across disease categories who did not respond, we confirmed previous data (Charles, Sabo et al. 1996) that BFU-E numbers correlated with response to IST. Of the 20 patients who grew 30 BFU-E and who were treated with IST, 18 (90%) responded. All of the patients in our cohort had BFU-E assay performed prior to treatment. An important difference between our study and that of Charles et al. was that all patients in their study met clinical criteria for PRCA. Our study included patients with cellular marrows and a variety of cytopenias but still all diseases whose primary therapy is thought to be IST. Furthermore, our study stratified the etiology of PRCA into LGL and non-LGL.

Not surprisingly, the BFU-E assay cannot always distinguish between MDS, systemic autoimmunity, LGL and PRCA. However, it appears to be a useful adjunct to more standard bone marrow diagnostic tests for assessing cytopenias such as cytogenetics and flow cytometry. The assay may be particularly useful in patients with cytopenias and a previously non-diagnostic cellular bone marrow assessment. Growth above the normal median (40 BFU-E per 10^5 mononuclear cells) always excluded MDS and was also associated with response to IST.

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Figure 1. Boxplot of B-FUE Growth By Diagnoses

This boxplot graphically displays the distribution of the BFU-E values for each disease category including the minimum and maximum values, the medians and the lower and upper quartiles. The dots represent outliers. The comparison between the median values of each disease was performed using the Kruskal- Wallis one-way analysis of variance with $p=0.0001$.

MDS Patients (N=17)

Table 1

Age (year) /Sex	WHO Diagnosis/IPSS	ANC (/ cu mm)	Hb (g/dL)	Retic (K/cu mm)	Plts (K/cu mm)	Cellularity (%)	Karyotype	FISH	CD34 (%)	BFU-E (10 ⁵ MMNC)	Clinical Follow up	Response To Therapy
76 M	RA/ Int-1	1245	8.6	28.2	212	50–70	NL	NL	0.7	5.00	Supportive care 5 mos, Deceased 2/2 disease	NA
71 M	RA/ Int-1	2800	7.7	33.8	86	100	NL	NL	NA	5.00	Supportive care 7 mos, Deceased 2/2 disease	NA
85 F	RAEB1/ Int-1	610	11.6	NA	47	Hypercell	NL	NL	0.17	34	Supportive care 6 mos	NA
67 F	tMN/NA	2040	13.4	53.4	32	90	Del11q23	11q23	NA	20.3	Supportive care 48 mos	NA
70 F	MDS-NOS/Int-1	9950	11.2	38.5	28	80	NL	NL	NA	5.70	Supportive care 20 mos	NA
70 M	RAEB1/Int-1	170	8.8 [*]	92.6	20	90	NL	NL	7.76	1.7	Growth factors, PR 6 mos; Relapse, AZA, NR 3 mos	NR
77 F	RA/Int-1	3260	11.1 [*]	6.9	261	30	NL	NL	NA	7.30	AZA, NR 18 mos	NR
71 M	RCMD/Int-1	26	6.9	11.1	43	40–50	NL	NL	0.21	38.00	AZA x 9 mos, NR 29 mos	NR
68 M	RAEB2/Int-1	700	8.7	2.4	26	90	NL	NL	NA	0.00	AZA, NR 6 mos; Trial, NR 6 mos; induction chemo, NR 6 mos; Deceased 2/2 disease	NR
60 F	CMML/ Int-1	4460	9.3	13	32	55	t(9, 19)	NL	NA	9	AZA x 2 cycles, NR 6 mos	NR
65 F	tMN/NA	2000	9	NA	27	50	Complex	–5, –7	NA	5.7	AZA, NR 3 mos; Deceased 2/2 disease	NR
62 M	RAEB2/Int-1	1870	10 [*]	43.1	25	80	NL	NL	NA	14.7	Lenalidomide NR 3 mos; AZA NR 3 mos; Deceased 2/2 disease	NR
56 F	tMN/ NA	900	8.8	NA	26	95	Complex	–5, –7	NA	4	Induction chemo, NR 6 mos	NR
71 M	RAEB2/ Int-1	500	11.5 [*]	163.3	84	70–80	NL	NL	4.19	0.67	Induction chemotx, NR 5 mos, Deceased 2/2 disease	NR
48 F	tMN/ NA	2830	11.1	20	75	60	Complex	–5, –7	NA	3.3	Induction chemo, NR 6 mos	NR
54 F	RAEB2/Int-2	83	8.5	72.9	104	60	NL	NL	NA	0.00	Induction Chemo, BMT, CR 12 mos	CR
55 F	RCMD/Int-1	1850	7.8	41.2	504	sternal	Complex	5q-	NA	2.70	Lenalidomide CR 20 mos	CR

Dx Diagnosis; WHO World Health Organization; IPSS International Prognostic Scoring System; RAEB Refractory Anemia with Excess Blasts; RA Refractory Anemia; RCMD Refractory cytopenias with multilineage dysplasia; tMN Treatment Related Myeloid Neoplasm; Int Intermediate;

^{*} transfused; Abnormal: ABNL; Normal NL; ABNL flow: Blasts, as distinguished by low density CD45 and low right angle scatter, are increased.; AZA: Azacitidine; AML: Acute myeloid leukemia; Tx: treatment; BMT: bone marrow transplantation; NL for PNH: The GPI anchor proteins are positive at levels greater than 0.01% on red cells, monocytes and granulocytes; supportive care: Growth factors and transfusions

Table 2

PRCA (not caused by LGL) (N=9)

Age (years)/Sex	Etiology	ANC (/cu mm)	Hb (g/dL)	Retic (K/cu mm)	Plts (K/cu mm)	Cellularity (%)	Flow	PNH	BFU-E (/10 ⁵ MMNC)	Clinical Follow up	Response to IST
45 F	Thymoma	3540	11.7*	6.8	225	50	NL	NA	12.00	Thymectomy+ adjuvant chemotx, CR 23 mos	NA
77 F	Antiepo Antibodies	4010	4.4	1.4	135	15	PRCA	NA	4.30	Supportive care 2 mos, Deceased 2/2 disease	NA
70 M	Idiopathic	5700	9 *	6.1	224	25	NL	NL	10.00	ATG/CsA, CR 24 mos; developed MDS s/p BMT, CR 20 mos	CR
29 F	Idiopathic	1690	9.7*	11.5	208	50 ^f	PRCA	NL	35.00	CY, NR 4mos; CsA, CR 12 mos	CR
35 F	ABO incompatible BMT	2990	10.8*	12.8	115	NA	NL	NL	23	Rituximab, PR 9 mos	PR
70 F	ABO incompatible BMT	2720	7.6*	5.8	207	80	NL	NL	24.30	Tacrolimus and rituximab, PR 9 mos	PR
59 M	Antiepo Antibodies	6270	7.1*	2.1	135	40	NL	NL	49.00	CY, CsA IVIg, PR 11 mos, Deceased from liver failure 2/2 Hepatitis C	PR
44 F	Thymoma	4080	8.4 *	61.7	260	55	PRCA	NA	18.70	Thymectomy, Intermittent compliance with CsA, Tacrolimus, ATG, NR 61 mos	NR
77 M	Idiopathic	3200	7.5*	4.3	159	25	PRCA	NL	52.70	CsA, NR 15 mos	NR

BMT Bone marrow transplant; CsA cyclosporin, ATG: antithymocyte globulin; PRCA: Absence of erythroid precursors; NL for PNH: The GPI anchor proteins are positive at levels greater than 0.01% on red cells, monocytes and granulocytes. Chemotx: chemotherapy with cisplatin, Adriamycin and prednisone.

All PRCA patients had normal FISH and karyotype.

^fCD34 % on performed on this patient 0.57%

Table 3

LGL (N=15)

Age (years)/Sex	ANC (/cu mm)	Hb (g/dL)	Retic (K/cu mm)	Plts (K/cu mm)	Cellularity (%)	CD34 count (%)	Flow	TCR	BFU-E (/10 ⁵ MMNC)	Clinical Follow up	Response to IST
Single Lineage Cytopenias (n=8)											
62 F	1230	8.9*	17	400	20	0.13	LGL	NA	20.00	CsA, CR 9 mos	CR
61 M	3760	7.8*	4	245	90	NA	LGL	Positive	23.50	CsA, CR 6 mos	CR
80 M	1400	6.5*	4.5	261	20	0.4	LGL	Positive	56.00	CsA, CR 13 mos	CR
63 M	2960	9.3*	4.7	259	40	NA	LGL	Positive	62.00	CY, CR 6 mos	CR
60 M	3380	7.1*	34	320	70	0.57	LGL	Positive	81.00	CY, CR 6 mos	CR
54 M	2670	7.7*	51.7	166	50	NA	LGL	Positive	84.70	CY, CR 26 mos	CR
26 F	0	13.8	2	235	90	1.6	LGL	Positive	132.70	growth factor, steroids, PR 24 mos; MTX, PR 12 mos; CsA, PR 9 mos; high dose IV CY, PR 9 mos	PR
23 M	1750	7.7*	3.3	164	75	0.31	LGL	Positive	21.00	MTX, NR 12 mos; CsA, NR 9 mos; Fludarabine, NR 4 mos; BMT, NR 6 mos Deceased 2/2 disease	NR
Multilineage cytopenias (n=7)											
74 M	580	8.5	1	228	40	NA	LGL	Positive	86.70	CsA, CR 13 mos	CR
32 M	1190	3.6	0	482	>70	0.58	LGL	Positive	105.00	CY, CR 6 mos; CsA, CR 28 mos	CR
31 F	290	11.1	0.6	371	65	NA	LGL	Positive	101.00	MTX, PR 15 mos	PR
52 M	300	10.7	3.7	44	50	0.12	LGL	Positive	24.70	IVIg/steroids, PR 9 mos	PR
60 M	945	8.8*	1.8	32	55	0.29	LGL	Positive	76.3	CY, PR 6 mos	PR
57 M**	490	11.6*	48	105	45	NL	3% LGL	Positive	25.00	CY, PR 6 mos, treatment held for 6 mos; diagnosed with MDS with increased blasts AZA x 6, PR 22 mos	PR
61 M	0	11.3	1.2	216	80	0.55	LGL	Positive	61.30	CsA, NR 12 mos	NR

LGL defined as population of CD3, dim CD5, CD8 and CD57 positive cell; MTX Methotrexate; CsA cyclosporine; IVIG Intravenous immunoglobulin

All LGL patients had normal FISH and karyotype.

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Patient had LGL at time the BFUE was obtained; later evolved to MDS.

Table 4

Systemic Inflammation (N=7)

Age (years)/Sex	Diagnosis	Inflammatory Marker	ANC/ ALC (/cu mm)	Hb (g/dL)	Retic (K/cu mm)	Plts (K/cu mm)	Cellularity	BFU-E (/10 ⁵ MMNC)	Clinical Follow up	Response to IST
42 F	SLE	ANA 1:160, positive APLA, Anticardiolipin	440/890	10.2	64.6	105	60	63.70	rituximab, CR 15 mos	CR
66 F	Evan's syndrome	ANA 1:640, Antiplt antibodies, Coombs negative	138/497	9.7	105.5	25	80	111.00	Prednisone, Rituximab, CR 32 mos; Relapse, rituximab, PR 1 month	CR
24 F	SLE	ANA 1:640, APLA, dsDNA, Coombs IgG+	1320/640	7.2*	14.3	140	90	136.70	Prednisone, plaquenil, CR 12 mos	CR
44 F	RP	Positive biopsy	2080/560	11.4	29	246	65	107.7	Prednisone, infliximab, CR 6 mos	CR
28 M	SLE	ANA 1:640, dsDNA	4188/480	8*	49	206	75	100.00	Prednisone, plaquenil, PR 12 mos	PR
63 M	Vasculitis	ANA 1:80, positive angiogram for vasculitis	670/110	7.6	39	88	60	49.00	Prednisone, CY, PR 8 mos	PR
31 F	SLE	ANA 1:640, Anti-Ro, positive Smith	3500/440	8.7*	16	41	70	76.0	Prednisone, MMF PR 6 mos	PR

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The specimen contains a mixture of cells. Blasts, as distinguished by low density CD45 and low right angle scatter, are not increased and cannot be distinguished from NLmarrow blasts. The myeloid maturation is phenotypically orderly. CY= oral Cyclophosphamide, MMF= mycophenolate SLE systemic lupus erythematosus RP Relapsing polychondritis; APLA Antiphospholipid antibodies

All systemic inflammation patients had normal FISH, flow cytometry, and karyotype.

Table 5

Cause of Death

Disease	Cause of Death	BFUE (/10⁵ MMNC)	Time from BFU-E performed (months)
LGL	Infection, 9 months s/p BMT for progression of LGL	21.00	31
PRCA-AntiEpo Abs	Underlying disease	4.30	1
PRCA-AntiEpo Abs	Hepatic failure from Hepatitis C and transfusion dependence	49.00	11
PRCA- idiopathic	Underlying disease	52.7	15
MDS	Underlying disease	5.00	5
MDS	Underlying disease	5.00	7
MDS	Underlying disease	0.67	9
MDS	Underlying disease	0.00	18
MDS	Underlying disease	14.7	5
MDS	Underlying disease	5.7	3

AntiEpo Abs: Anti-erythropoietin antibodies