


Long-term immunosuppression and multiple transplants predispose systemic lupus erythematosus patients with cytopenias to hematologic malignancies

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Abstract

Cytopenias in systemic lupus erythematosus (SLE) require clinical and laboratory workup and bone marrow (BM) examination to determine the cause and for appropriate patient management. Common causes include an increase in SLE activity, immune-mediated hemolysis, iron deficiency, antiphospholipid antibody syndrome, infection, or the effect of medications. We retrospectively evaluated the clinical and laboratory findings of patients with SLE and cytopenias who had undergone BM studies to determine the indicators of malignancy.

We retrospectively reviewed medical records of patients with SLE who presented with cytopenias for their disease course, medications, laboratory parameters and documented the spectrum of morphological changes in BM including CD34 expression.

Twenty patients with SLE had undergone BM biopsy for evaluation of cytopenias. 14/20 (70%) of the patients had reactive BM, and the rest had hematologic malignancies involving the BM. Of these 14 patients, 8 had hypocellular marrow with loss of precursor cells (low CD34), 4 had left shift in myeloid lineage, 3 had serous atrophy, and 1 had multilineage dysplasia. The 6 patients with hematologic malignancies included 2 with diffuse large B cell lymphoma, and one each of natural killer/T cell lymphoma, post-transplant lymphoproliferative disorder, Hodgkin lymphoma, and myelodysplastic syndrome evolving to acute myelogenous leukemia. The presence of autoantibodies, SLE activity, and lupus nephritis were comparable in patients with and without neoplasia. However, the duration of the use of multiple immunosuppressants, years since renal transplant (22 vs 10), multiple transplants, and the presence of other autoimmune diseases were greater in those with neoplasia. Two of the 14 patients with non-neoplastic BM and 1 with the neoplastic BM had nonhematological malignancy.

Clinical and laboratory findings, the number of transplants, and the use of immunosuppressive agents can guide physicians to identify patients with a higher risk of developing hematologic malignancy. BM findings of cytopenia in SLE are often due to increased disease activity causing global cell death and dysmaturation. SLE patients presenting with cytopenias, with a history of long-term exposure to immunosuppressive drugs, should be regularly screened for hematologic and nonhematologic malignancies.

Abbreviations: anti-dsDNA = anti-double stranded DNA, aPL = anti-phospholipid antibodies, AZA = azathioprine, BM = bone marrow, Hgb = hemoglobin, MDS = myelodysplastic syndrome, MTX = methotrexate, NHL = non-Hodgkin lymphoma, SLE = systemic lupus erythematosus.

Keywords: autoimmune disease, bone marrow, immunosuppression, malignancy, neoplasia, systemic lupus erythematosus

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The study was reviewed and approved by the Institutional Review Board & Privacy Board (IRB) of the State University of New York, Downstate Health Sciences University (873792-1). The IRB granted ethical approval and waiver of consent to access the patient database.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with a predilection for women of childbearing age with a considerable variation in number and severity of organ involvement. The hallmarks of SLE are microvascular inflammation and the generation of autoantibodies, particularly anti-nuclear antibodies. Hematological disorders, including anemia, leukopenia, lymphopenia, and thrombocytopenia are part of the 1982 revised American College of Rheumatology and 2012 Systemic Lupus International Collaborating Clinics classification criteria for SLE.^[1,2]

Hematological abnormalities are a significant source of morbidity and mortality in SLE. These abnormalities can be related to the pathophysiology of SLE, its sequelae, or the agents employed in its management.^[3] Anemia encountered in up to 63% of the SLE patients could be secondary to anemia of chronic disease, autoimmune hemolytic anemia, microangiopathic hemolytic anemia, pure red cell aplasia, or aplastic anemia.^[3,4] Neutropenia, present in 50% of the patients, has been postulated to occur from autoimmune mechanisms leading to increased destruction of granulocytes by anti-neutrophil antibodies, increased margination, and decreased the number of colony-forming units due to T lymphocyte suppression.^[3,5,6]

Patients with active or severe SLE frequently present with lymphopenia with T cell numbers affected more than those of B cells.^[7] Both IgG and IgM anti-lymphocyte antibodies are identified in patients with lymphopenia. There is increased apoptosis in lymphocytes in patients with SLE than those in healthy controls.^[8] The presence of lymphopenia may be clinically silent or associated with an increased risk of infection and/or active SLE. Fever, polyarthritis, as well as central and peripheral nervous system disease, in particular, are associated with lymphopenia.^[9]

Thrombocytopenia is mostly due to anti-platelet antibody-mediated destruction of platelets in peripheral blood; however, sequestration and diminished production of platelets in the bone marrow (BM) also play an essential role. Thrombocytopenia can also be due to the interaction between anti-phospholipid antibodies (aPL) and platelet-antigen antibodies.^[3] The presence of aPL confers a 4-fold risk of thrombocytopenia.^[10] Thrombocytopenia, as an independent risk factor for early mortality in SLE, has also been documented.^[11]

Increased risk of cancer among SLE patients has also been reported. The standardized incidence ratio was 2.75 for all hematologic malignancies and 3.64 for non-Hodgkin lymphoma (NHL). Furthermore, the risk was also higher for lung and hepatobiliary malignancies.^[12] In addition, SLE patients tend to have high-grade lymphomas that run an aggressive course; SLE disease activity, presence of hematologic abnormalities, Epstein-Barr virus infection, increased B cell activation, and proliferation are associated with increased lymphoma risk.^[13]

At some point, SLE patients with hematologic manifestations undergo BM biopsy for the exclusion of lymphoproliferative disorder and/or drug toxicity in the BM. However, the guidelines for BM biopsy in SLE patients are not clearly defined, and the decision to biopsy depends on the clinical acumen of the physician. Very few studies have tried to correlate the relation between clinical, histologic, laboratory, immunologic workup, and medication history with the development of hematologic manifestations.

Our aim was to evaluate the clinical and laboratory parameters of SLE patients who had undergone BM biopsy for the evaluation

of cytopenia and to determine the indicators that could help predict hematologic malignancy as a cause of cytopenia. We also evaluated the spectrum of morphological changes in the BM due to SLE activity and/or immunosuppressive therapy.

2. Patients and methods

The laboratory information system and medical records were searched for patients, 18 years and older, diagnosed with SLE as per the 2012 Systemic Lupus International Collaborating Clinics diagnostic criteria, who had undergone BM aspiration and/or biopsy for hematologic abnormalities (Fig. 1). Patient demographics, clinical and laboratory findings, medication history, indications for BM biopsy, BM biopsy findings, and outcomes were recorded. Laboratory findings and medication history at -12 , -9 , -6 , -3 , 0 , $+3$, $+6$, $+9$ and $+12$ months relative to the time of BM biopsy (0) were noted. Patients with active parvovirus B19, Epstein-Barr virus, and cytomegalovirus infection were excluded. Those who underwent a BM biopsy as part of staging workup for an extramedullary lymphoma were also excluded. Indications for BM studies included cytopenias (anemia, leukopenia, thrombocytopenia, pancytopenia) suspected lymphoma (post-transplant lymphoproliferative disorder, multiple myeloma) and lymphadenopathy (Fig. 1).

BM slides were retrieved, re-examined before and after immunostaining for CD34 (Ventana Medical Systems Tucson, AZ). BM aspirates stained with Wright–Giemsa and BM biopsies with hematoxylin and eosin, and iron stains were evaluated. Histopathological data were assessed for cellularity, myeloid, erythroid and megakaryocytic dysplasia, abnormal localization of myeloid precursors, blasts, lymphoid follicles, plasma cells, and iron, and flow cytometric data were reviewed.

The study was reviewed and approved by the Institutional Review Board & Privacy Board (IRB) of the State University of New York, Downstate Health Sciences University (873792-1). The IRB granted ethical approval and waiver of consent to access the patient database.

Statistical analyses were performed using SPSS (v. 26). We used measures of central tendencies and dispersion for continuous variables and frequency distribution for categorical variables. Data are presented as mean \pm SEM as well as a cross-tabulation format for categorical variables. Numerical data for patients with neoplastic and non-neoplastic BM were compared by Mann–Whitney *U* test for continuous variables and 2-way ANOVA.

3. Results

Twenty patients with SLE who had had BM studies were identified. Two (10%) of the patients were males, and 18 (90%) were females. Fifteen (75%) of the 20 patients were Black, and 5 (25%) were White. The mean age at SLE diagnosis was 25.8 (range: 21–74) years. Patients with hematologic BM malignancy had a mean age of 44.5 (range: 31–74) years, and those with non-neoplastic BM had a mean age of 45.6 (range: 21–75) years at the time of BM biopsy. The mean duration of SLE at the time of BM biopsy for patients with malignancy was 19.5 (range: 6–32) years and for those without malignancy was 17.2 (range: 0–35) years (Table 1).

Four of twenty patients (20%) had undergone a renal transplant, and 3 of them had received >2 transplants; 2 of the latter developed hematologic neoplasia. Patients with neoplastic BM had more years elapsed after renal transplant

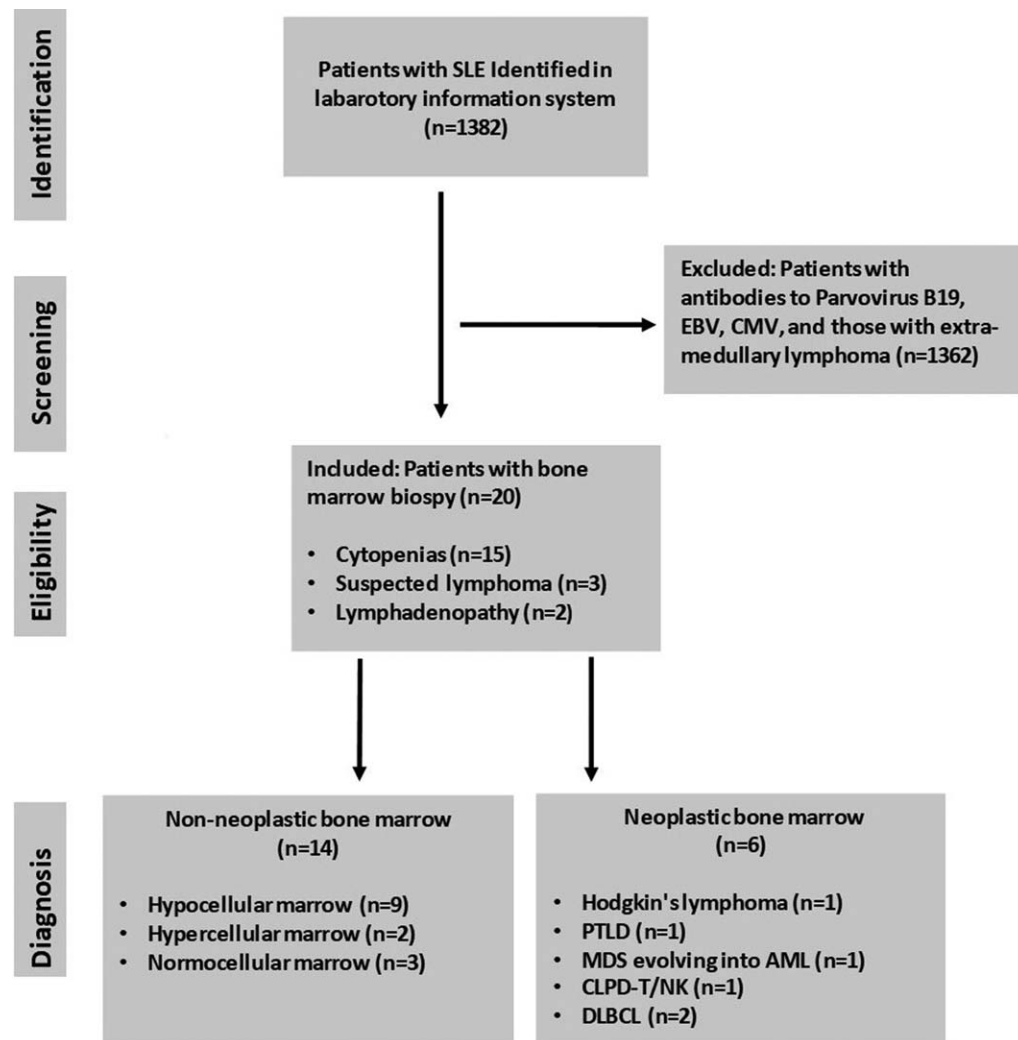


Figure 1. Flow chart showing selection of patients with systemic lupus erythematosus who had bone marrow biopsy to diagnose or rule out hematologic malignancy. AML = acute myeloid leukemia, CLPD-T/NK = chronic lymphoproliferative disorder, CMV = cytomegalovirus, DLBCL = diffuse large B cell lymphoma, EBV = Epstein-Barr virus, MDS = myelodysplastic syndrome, PTLD = post-transplant lymphoproliferative disorder, SLE = systemic lupus erythematosus.

Table 1

Demographic and clinical features of patients with systemic lupus erythematosus (SLE) who had undergone bone marrow (BM) studies.

Demographic and clinical features of patients	Bone marrow		Total (n=20)
	Non-neoplastic (n=14)	Neoplastic (n=6)	
Gender			
Female	12	6	18 (90%)
Male	2	0	2 (10%)
Race			
Black	11	4	15 (75%)
White	3	2	5 (25%)
Age at SLE diagnosis, mean (range) in yr	17 (21–75)	20 (31–74)	26 (21–75)
Age at BM biopsy, mean (range) in yr	46 (21–75)	45 (31–74)	42 (21–75)
SLE duration at BM biopsy, mean (range) in yr	17 (0–35)	20 (6–32)	18 (0–35)
Renal transplant recipient	2 (14%)	2 (33%)	4 (20%)
Multiple renal transplants (≥2)	1 (7%)	2 (33%)	3 (20%)
Time since 1st transplant (yr)	10	22	
Other autoimmune disease	2 (14%)	2 (33%)	4 (20%)
Lupus nephritis Class IV/V	9 (69%)	4 (66%)	13 (68%)
Active lupus nephritis	4 (28%)	1 (16%)	5 (26%)

Table 2
Autoantibodies and complement (C3) in SLE patients with non-neoplastic and neoplastic bone marrow.

Bone Marrow	Patient	Immune profile						
		Anti-nuclear	Anti-dsDNA	Anti-Smith	Anti-SSA (Ro)	Anti-SSB (La)	Anti-RNP	Complement 3
Non-neoplastic	1	+	−	−	−	−	−	↓
	2	+	+	−	−	−	+	↔
	3	+	+	+	+	+	+	↓
	4	+	−	−	−	+	−	↔
	5	−	−	−	NA	NA	NA	↔
	6	+	+	−	NA	NA	NA	↔
	7	+	+	−	+	+	−	↓
	8	+	+	+	+	+	+	↓
	9	+	+	NA	NA	NA	NA	↔
	10	+	+	NA	NA	NA	NA	↓
	11	+	+	NA	−	−	NA	↔
	12	+	+	+	NA	NA	NA	↓
	13	+	+	−	−	−	−	↔
	14	+	+	+	−	+	+	↓
Neoplastic	15	+	−	−	−	−	−	↓
	16	−	+	−	−	−	−	↔
	17	+	+	+	−	+	+	↓
	18	+	+	+	+	−	+	↔
	19	+	+	−	+	−	+	↓
	20	+	+	−	−	+	+	↓

+ = detected, ↑ = increase, ↓ = decrease, ↔ = normal, − = not detected, NA = not available.

(22 vs 10 years). Four patients (2 in each of the neoplastic BM and non-neoplastic BM groups) had another autoimmune disease in addition to SLE (3 had rheumatoid arthritis and 1 had Sjogren syndrome). Biopsy-proven lupus nephritis class IV or V was present in 13 (68%) of all patients with equal frequency in both groups of patients; patients with non-neoplastic BM had a higher frequency of active lupus nephritis at the time of BM biopsy (28% vs 17%) (Table 1).

The most commonly detected antibodies in our patients, in their disease course, were anti-nuclear antibodies (90%) and anti-double stranded (DNA) anti-dsDNA (70%), while others (anti-Smith, anti-Sjögren-syndrome-related antigen A, anti-Sjögren syndrome type B, anti-ribonucleoprotein) were detected in about 1/3 of all patients. 50% of patients with both neoplastic and non-neoplastic BM had detectable anti-dsDNA at the time of BM biopsy. Patients with neoplasia had a higher frequency of chronic low complement levels than those with non-neoplastic BM (50% vs 35%) (Table 2).

There were no significant differences in hematologic parameters (white blood cells, Hgb, platelets) of patients with or without neoplastic disease from −12 months to −6 months, however, at −3 months there was considerable drop in leukocytes, hemoglobin (Hgb), platelets, neutrophils, and lymphocytes in patients with neoplasia as compared to those without neoplasia. Patients with non-neoplastic BM had higher creatinine and urea nitrogen concentrations and low glomerular filtration rate than those with neoplastic BM (Fig. 2).

BM was non-neoplastic in 14 patients, while 6 had primary hematologic BM malignancies. Among the 14 patients with non-neoplastic BM, 8 (57%) showed hypocellular marrow, and 6 (42%) showed normocellular to mildly hypercellular marrow. Erythroid hyperplasia was present in 5 (35%) patients; the rest had normocellular erythroid lineage. Myeloid cell lineage was left shifted in 4 (30%) patients. Megakaryocytes were morphologically small, naked, and hypolobated in all SLE patients. Dysplasia

involved mostly erythroid precursor cells and included binucleate cells, megaloblastic change, dyssynchrony, and nuclear budding, involving 10% to 25% of the cells. Multilineage dysplasia was observed in 9 (64%) patients. There was a decrease in CD34+ precursor cells in 4 (28%) patients. Serous atrophy replacing part or total BM space was observed in 3 (23%) of the BM biopsies. Iron was increased in 10 (76%) as determined by special staining. Lymphoid aggregates were seen in 3 (23%) patients. Two patients had 5% plasma cells, but no monoclonal spike was found on serum electrophoresis performed at the time of BM biopsy, and at 24 months subsequently (Table 3; Fig. 3).

Of the 20 SLE patients, 9 (45%) developed at least 1 malignancy in their lifetime. 6 were hematologic malignancies diagnosed by the BM biopsy. Two patients with non-neoplastic BM had a diagnosis of other carcinomas; verrucous squamous cell carcinoma (−13 years) in 1, and colon carcinoma (+18 months after the BM biopsy) in another patient who died subsequently. One patient with hematologic malignancy had a history of anal squamous cell carcinoma (−7 years) who also died subsequently during follow up. Of the 6 primary hematologic malignancies identified, we diagnosed large B cell lymphoma in 2 patients, and NK/T-cell lymphoma, post-transplant lymphoproliferative disorder, Hodgkin lymphoma, and myelodysplastic syndrome evolving to acute myeloid leukemia (AML) in 1 patient each, respectively.

Patients had been managed with steroids, hydroxychloroquine, mycophenolate mofetil, cyclophosphamide (Euro-regimen), tacrolimus, and methotrexate (MTX), as single drugs or in combination over their disease course. Patients with neoplastic BM were more likely to be on multiple immunosuppressants than those with non-neoplastic BM (67% vs 43%). When comparing medication between the 2 groups of patients, no differences were found for hydroxychloroquine, MTX, or tacrolimus. Immunosuppressants [azathioprine (AZA), biologics] were more frequently used in patients who developed hematologic disease.

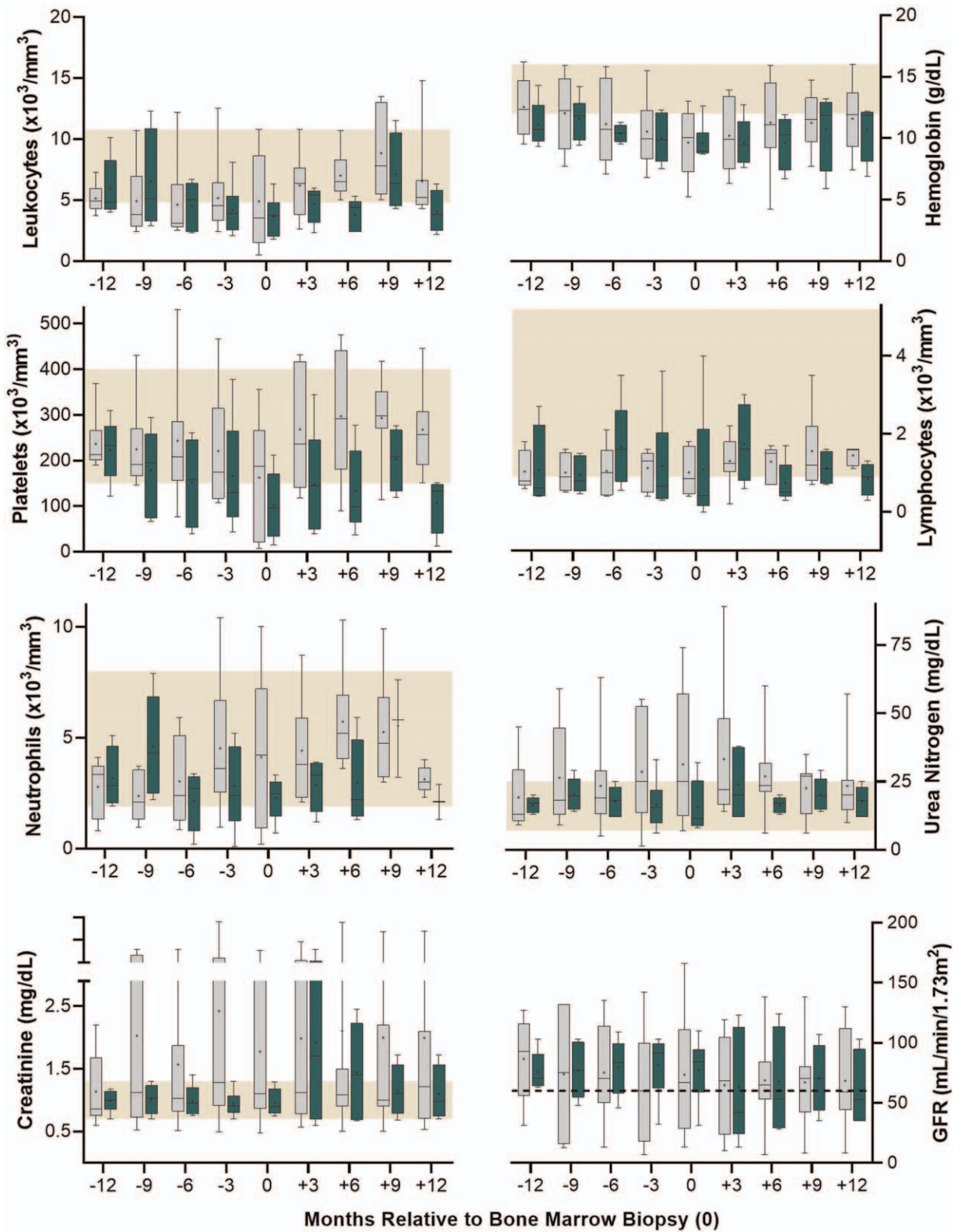


Figure 2. Leukocyte, hemoglobin, platelet, lymphocyte, neutrophil, urea nitrogen, creatinine levels, and glomerular filtration rate at -12, -9, -6, -3, 0, +3, +6, +9, +12 months relative to bone marrow biopsy (0) of SLE patients with (green) and without (grey) neoplasia. Bars indicate 5th and 95th percentiles; boxes represent the 25th and 75th percentiles; lines inside the boxes are medians, and asterisks indicate means. Shaded background represents reference ranges. Broken line denotes the reference value for glomerular filtration rate (GFR) at 60 mL/min/1.73m².

Table 3**Features of bone marrow of patients without neoplastic disease.****Bone marrow findings in non-neoplastic patients**

Patient	Cellularity	Serous atrophy	Myeloid	Erythroid	Megakaryocyte	Dysplasia	CD34	Iron*	Lymphoid aggregates
1	Hypocellular	+	↓	↔	SNH	No dysplasia	↓	NA	—
2	Hypercellular	—	↔	↔	↑	No dysplasia	↔	1	—
3	Hypocellular	—	LS	Hyperplasia	SNH, ↑	BN, megaloblastoid	—	3	—
4	Normocellular	—	↔	↔	SNH	Dyssynchrony	NA	0	—
5	Hypocellular	—	LS	Hyperplasia	SNH, ↑	BN, megaloblastoid	↑	3	—
6	Hypercellular	+	↔	↔	SNH, ↑	No dysplasia	↔	4	—
7	Hypocellular	+	LS	Hyperplasia	SNH, ↑	BN, budding, dyssynchrony	—	3	—
8	Hypocellular	—	↓, LS	↔	SNH, ↓	Dysplasia, megaloblastoid	—	3	+
9	Hypercellular	NA	NA	NA	NA	No dysplasia, 6% clonal plasma cells	NA	3	NA
10	Hypocellular	—	↓	↔	SNH	Nuclear budding	↓	3	+
11	Hypocellular	—	↓	Hyperplasia	SNH	Dyssynchrony	↓	3	—
12	Normocellular	—	↓	↔	SNH	BN, megaloblastoid	↓	0	—
13	Hypocellular	—	↓	↔	SNH	No dysplasia	↔	2	—
14	Normocellular	—	↔	Hyperplasia	SNH	Irregular nuclear, binucleate	↔	0	+

+ = present, ↑ = increase, ↓ = decrease, ↔ = normal, = absent, BN = binucleate, LS = left shift, NA = not available, SNH = small naked hypobulbated.

* Iron staining intensity.

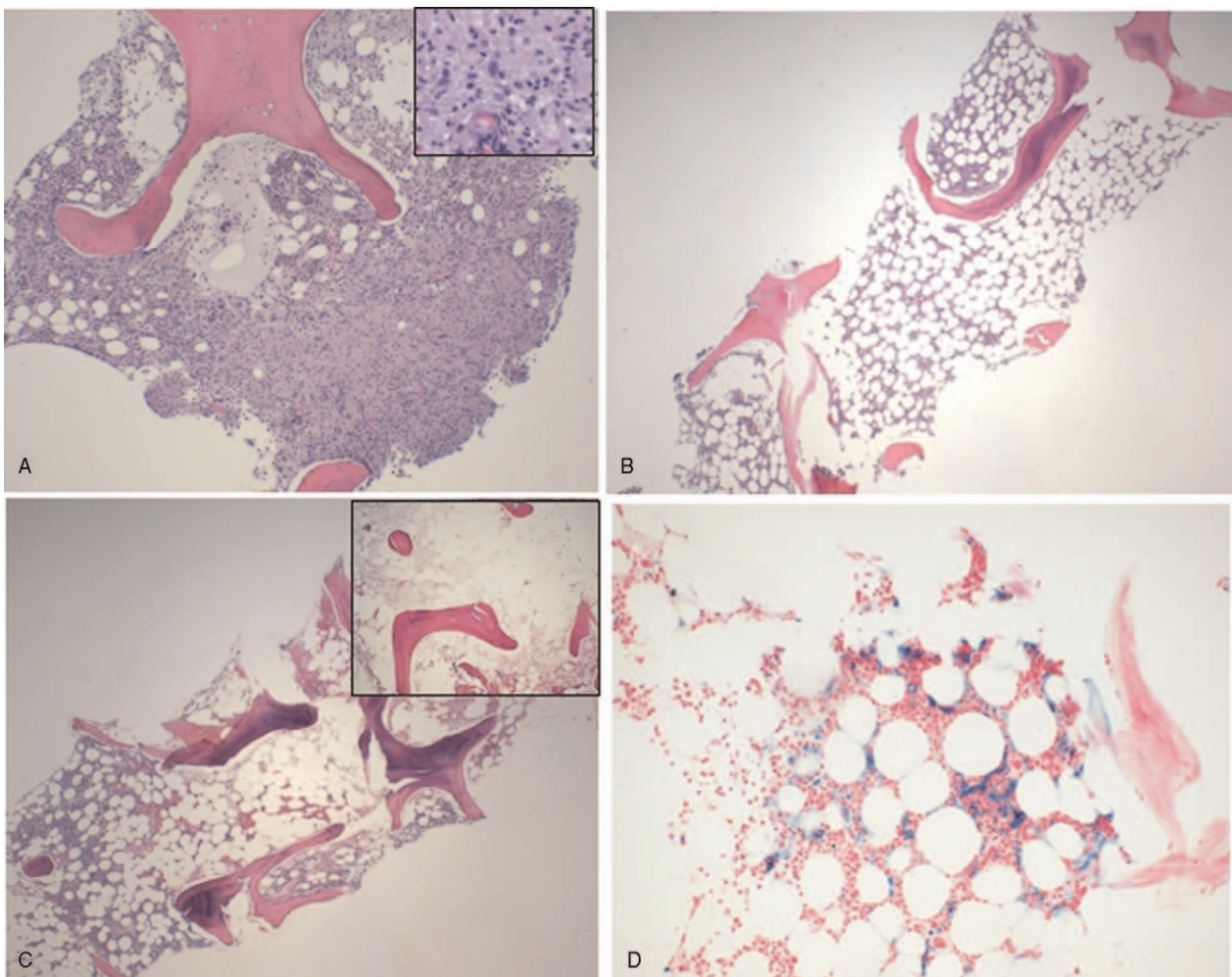


Figure 3. Representative photomicrographs of bone marrow biopsy in patients with SLE. (A) Hodgkin lymphoma (H&E, ×100). Inset, Reed-Sternberg cell (H&E, ×1000). (B) Hypocellular marrow (H&E, ×40). (C) Serous atrophy (H&E, ×40). Inset showing replacement of bone marrow elements with glycosylated material (H&E, ×100). (D) Increased storage iron in the bone marrow (Iron stain, ×100).

Table 4
Medication history of SLE patients with non-neoplastic and neoplastic bone marrow for 6 months prior to the bone marrow biopsy.

Bone Marrow	Patient	Medications								
		Azathioprine	Hydroxychloroquine	Leflunomide	Methotrexate	Mycophenolate	Orencia	Prednisone	Rituximab	Tacrolimus
Non-neoplastic	1					+		+		+
	2		+	+				+		
	3		+					+	+	
	4		+	+	+			+		
	5		+					+		
	6					+				+
	7	+						+		
	8		+				+	+		
	9		+					+		
	10									
	11	+	+					+		
	12							+		
	13					+				
	14									
Neoplastic	15							+		+
	16							+		+
	17		+		+			+		
	18	+	+			+		+		
	19	+						+	+	
	20		+		+		+	+		

Patients with non-neoplastic BM findings responded to the reduction of immunosuppression, steroids, granulocyte-colony stimulating factor and erythropoietin (EPO) (Table 4).

Overall survival was measured from 5 to 13 years. In the non-neoplastic BM group, 1 patient died of metastatic colon carcinoma diagnosed 20 months post BM biopsy; 5 of 14 patients were lost to follow-up. In the neoplastic BM group, 1 of the 6 patients died of AML developing from myelodysplastic syndrome (MDS). One patient with T cell lymphoma was lost to follow up.

The presence of antibodies, indicators of SLE activity and lupus nephritis are comparable in the 2 groups, however, more prolonged immunosuppressive therapy, years postrenal transplant (22 vs. 10 years), history of multiple transplants and the occurrence of other autoimmune disease were higher in patients with neoplasia. Exposure to >2 immunosuppressive drugs (AZA and biologics) increased association with malignancy. Of the hematological findings: leukopenia, neutropenia, lymphopenia, thrombocytopenia, and serological findings: chronic low complement levels were seen more frequently in patients with neoplastic BM. Renal function was abnormal in the non-neoplastic group (glomerular filtration rate <60, elevated creatinine and urea nitrogen). BM was mostly hypocellular with low hematopoietic precursor cells. Dysplastic changes were frequent in erythroid lineage cells and megakaryocytes but there was no increase in lymphocytes and plasma cells.

4. Discussion

SLE is an autoimmune disorder that virtually affects every organ system of the body. There is an overall increased risk of both hematological and non-hematological malignancy in SLE, with NHL being the most common.^[12] While every component of the blood and coagulation cascade can be affected in SLE, the most common hematological abnormalities are lymphopenia, neutropenia, anemia, thrombocytopenia, and aPL syndrome.^[3,14] Since

these hematological abnormalities have been found to occur in SLE independent of disease severity,^[15] these should be attended to promptly with a BM biopsy to rule out a neoplastic process. There is a paucity of information on the relationship between clinical and laboratory findings and medication history with the development of hematologic neoplasms. We aimed to identify the clinical and laboratory findings which can likely help ascertain whether the cytopenias are the result of a hematologic neoplasm or part of the SLE disease spectrum.

BM alterations in SLE have been the focus of a number of studies since the early 1950s when 32 of 111 SLE patients with hematological abnormalities underwent BM biopsy without revealing a plausible explanation for the observed cytopenias.^[16] Subsequent reports have described hypocellularity, increased reticulin, myelofibrosis and necrosis, abnormal iron stores, and increased plasma cells among other abnormalities.^[17-23] Dyserythropoiesis and hypoplasia have been found in the BM of 40% of the SLE patients, and gelatinous or serous transformation have been observed in fewer than 8% of the SLE patients.^[24,25]

Consistent with previous reports, we observed hypocellularity and myeloid hypoplasia in 57% of the patients with non-neoplastic BM, however, serous atrophy was found at a higher frequency (23 vs 8%) than that reported previously.^[26] Global hypocellularity, myeloid hypoplasia and necrosis are notable findings in the BM of SLE patients with cytopenia in our study. Increased lymphoid aggregates and plasmacytosis have been observed in the BM of SLE patients.^[19,24,27] These features were especially prominent when BM was biopsied at the time of active lupus nephritis with increased disease activity and elevated levels of anti-dsDNA antibodies.^[28] We rarely observed lymphoid aggregates and plasmacytosis. We could not confirm this association as the only patient who had BM plasmacytosis had neither lupus nephritis nor anti-dsDNA antibodies. CD34 analysis of hypocellular BM revealed varying degrees of reduction in the number of hematopoietic precursors (6/7) which likely results in global hypocellularity observed in the BM of SLE

patients. Such reduction in the number of hematopoietic stem cells in SLE patients has also been documented previously.^[29] Increased iron stores found in 76% of our patients, and also reported previously,^[19] may indicate the chronic nature of the disease and the inability of erythroid lineage to utilize the iron for hematopoiesis. The erythroid and megakaryocytic lineage cells showed dysplasia in 64% of our patients; it is not uncommon to find dysplasia in all cell lineages in SLE patients and it is, in fact, reversible during the course of the disease and is a sign of disease severity.^[15,24,30]

We observed hematological malignancy in 30% of our patients with SLE and pancytopenia; 2 of these patients developed diffuse large B cell lymphoma, and both received conventional chemotherapy with a good response. Other patients with lymphoma included a patient with natural killer/T cell lymphoma who had received 2 renal transplants, a patient with post-transplant lymphoproliferative disorder who had 3 renal transplants, and a patient with Hodgkin's lymphoma without a history of transplant. Exposure to >2 or more immunosuppressive drugs showed a tendency towards developing a hematologic malignancy. Biological markers for disease activity like hypocomplementemia, anti-dsDNA, and lupus nephritis were similarly present in patients with or without neoplastic marrow. Hematologic malignancy has been recognized as a sequela of SLE. Recent meta-analyses have shown a 5-fold greater risk for NHL and a 2-to-3-fold greater risk for Hodgkin lymphoma and leukemia among SLE patients.^[31,32] Another study of 16,409 patients at 30 different centers demonstrated a significantly increased risk for all hematologic malignancies in SLE (standardized incidence ratio: 3.02).^[33] Amongst the non-hematologic malignancies in our study, 2 patients had squamous cell carcinoma (skin and anal) and 1 had metastatic colonic adenocarcinoma, comprising 15% incidence. These data are consistent with the widely accepted higher risk of malignancy among SLE patients, especially for non-melanoma skin cancers.^[31,33,34]

The higher incidence of NHL has been postulated to derive from the combination of etiologies in SLE including chronic inflammation seen in autoimmune diseases, translocation of an oncogene next to a gene that mediates immune function and frequent exposure to cytotoxic medications which upregulate lymphocyte proliferation.^[31] Exposure to various types of antigens (e.g., autoantigens, viral and other microbial antigens) and/or activation of the B cell receptor signaling pathway leads to the development of lymphoma in these patients.^[35] B cell itself undergoes several genetic events during B cell maturation, which increases the chances of genetic aberration. Due to the autoimmune-inflammatory nature of SLE, B and T cells are exposed to and get activated by a range of antigens, leading to proliferation, clonal expansion, and accumulation of aberrant genetic events and development of lymphomas.^[13] It is hypothesized that the development of lymphoma in chronic inflammatory disease is heavily dependent on disease duration and disease activity. Immunosuppressive therapy may not be the primary driving factor for malignancy, but it appears to contribute to the increased risk of hematological malignancy.^[34,36–38]

Multiple treatment agents in SLE have been linked to lymphoproliferative disorders including MTX and cyclophosphamide.^[36,39,40] SLE patients treated with AZA are at an increased risk of developing myeloid neoplasms, especially MDS or AML.^[41–43] The only patient in our study who developed MDS/AML was also being treated with AZA. Mycophenolate mofetil and tacrolimus, used in transplant medicine, appear to

increase the risk of non-melanomatous skin cancer, squamous cell carcinoma, and basal cell carcinoma.^[44] Two patients in our study with squamous cell carcinoma did not have a history of using mycophenolate or tacrolimus.

Our study had limitations that include a small sample size and limited details of SLE disease activity indexes at the time of the diagnosis of hematological abnormality. Survival data are not available for 5 patients who were lost to follow-up. Most of our patient population is Black; thus, race comparison for the incidence of malignancy is not optimal.

5. Conclusions

We have found that the reliable indicators of underlying hematologic malignancy in a patient with SLE presenting with cytopenia are history of multiple transplants and long duration of immunosuppression. Patients receiving AZA and biologics were more likely to develop hematologic malignancies. Decreases in hematologic parameters (white blood cells, neutrophils, lymphocytes, platelets, Hgb) were more notable at 3 months prior to the BM biopsy in the neoplastic group. Other parameters like disease duration, disease activity, patient age, concurrent autoimmune diseases, and serological markers did not show any difference between these 2 groups. BM in SLE patients was mostly hypocellular with a decrease in progenitor cells and dysplastic changes in erythroid and megakaryocytic lineages, indicating disease activity. There was no increase in plasma cells or lymphocytes.

Clinicians treating patients with SLE presenting with cytopenia should take into account the number of consecutive transplants, length of immunosuppression, presence of biologics and AZA, and a recent drop in hematologic parameters to suspect the presence of hematologic neoplasm in these patients. The cause of pancytopenia in SLE patients appears to be a reduction of progenitor cells in the BM, causing hypocellularity and dysplastic changes, both of which are reversible. Although clinical and laboratory findings may guide clinicians, BM biopsy is essential for the definitive diagnosis or exclusion of a hematologic neoplasm.

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References

- [1] Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
- [2] Petri M, Orbai AM, Alarcon GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;64:2677–86.
- [3] Fayyaz A, Igoe A, Kurien BT, et al. Haematological manifestations of lupus. *Lupus Sci Med* 2015;2:e000078.
- [4] Aleem A, Al Arfaj AS, Khalil N, et al. Haematological abnormalities in systemic lupus erythematosus. *Acta Reumatol Port* 2014;39:236–41.

- [5] Starkebaum G, Price TH, Lee MY, et al. Autoimmune neutropenia in systemic lupus erythematosus. *Arthritis Rheum* 1978;21:504–12.
- [6] Yamasaki K, Niho Y, Yanase T. Granulopoiesis in systemic lupus erythematosus. *Arthritis Rheum* 1983;26:516–21.
- [7] Bhuyan UN, Malaviya AN. Deficiency of T cells in blood and tissues and severity of infections in systemic lupus erythematosus. *Indian J Med Res* 1978;67:269–78.
- [8] Silva LM, Garcia AB, Donadi EA. Increased lymphocyte death by neglect-apoptosis is associated with lymphopenia and autoantibodies in lupus patients presenting with neuropsychiatric manifestations. *J Neurol* 2002;249:1048–54.
- [9] Keeling DM, Isenberg DA. Haematological manifestations of systemic lupus erythematosus. *Blood Rev* 1993;7:199–207.
- [10] Abu-Shakra M, Gladman DD, Urowitz MB, et al. Anticardiolipin antibodies in systemic lupus erythematosus: clinical and laboratory correlations. *Am J Med* 1995;99:624–8.
- [11] Jung J-H, Soh M-S, Ahn Y-H, et al. Thrombocytopenia in systemic lupus erythematosus. *Medicine (Baltimore)* 2016;95:e2818.
- [12] Bernatsky S, Boivin JF, Joseph L, et al. An international cohort study of cancer in systemic lupus erythematosus. *Arthritis Rheum* 2005;52:1481–90.
- [13] Baecklund E, Smedby KE, Sutton LA, et al. Lymphoma development in patients with autoimmune and inflammatory disorders—what are the driving forces? *Semin Cancer Biol* 2014;24:61–70.
- [14] Bashal F. Hematological disorders in patients with systemic lupus erythematosus. *Open Rheumatol J* 2013;7:87–95.
- [15] Löfström B, Backlin C, Sundström C, et al. Myeloid leukaemia in systemic lupus erythematosus a nested case control study based on Swedish registers. *Rheumatology (Oxford)* 2009;48:1222–6.
- [16] Michael SR, Vural IL, Bassen FA, et al. The hematologic aspects of disseminated (systemic) lupus erythematosus. *Blood* 1951;6:1059–72.
- [17] Burkhardt R. The bone marrow in systemic lupus erythematosus. *Semin Hematol* 1965;2:29–46.
- [18] Daly HM, Scott GL. Myelofibrosis as a cause of pancytopenia in systemic lupus erythematosus. *J Clin Pathol* 1983;36:1219–22.
- [19] Pereira RM, Velloso ER, Menezes Y, et al. Bone marrow findings in systemic lupus erythematosus patients with peripheral cytopenias. *Clin Rheumatol* 1998;17:219–22.
- [20] Voulgarelis M, Giannouli S, Tasidou A, et al. Bone marrow histological findings in systemic lupus erythematosus with hematologic abnormalities: a clinicopathological study. *Am J Hematol* 2006;81:590–7.
- [21] Hunt KE, Salama ME, Sever CE, et al. Bone marrow examination for unexplained cytopenias reveals nonspecific findings in patients with collagen vascular disease. *Arch Pathol Lab Med* 2013;137:948–54.
- [22] Chalayer E, Ffrench M, Cathebras P. Bone marrow fibrosis as a feature of systemic lupus erythematosus: a case report and literature review. *Springerplus* 2014;3:349.
- [23] Mistry J, Knee G, Jayakar V. Systemic lupus erythematosus presenting to haematology with pancytopenia and features of macrophage activation syndrome. *BMJ Case Rep* 2018;2018: doi:10.1136/bcr-2017-222096.
- [24] Feng CS, Ng MH, Szeto RS, et al. Bone marrow findings in lupus patients with pancytopenia. *Pathology* 1991;23:5–7.
- [25] Yamamoto M, Belmont HM, Utsunomiya M, et al. Gelatinous transformation of the bone marrow in systemic lupus erythematosus. *Lupus* 2009;18:1108–11.
- [26] Wanitpongpun C, Teawtrakul N, Mahakkanukrauh A, et al. Bone marrow abnormalities in systemic lupus erythematosus with peripheral cytopenia. *Clin Exp Rheumatol* 2012;30:825–9.
- [27] Rosenthal NS, Farhi DC. Bone marrow findings in connective tissue disease. *Am J Clin Pathol* 1989;92:650–4.
- [28] Park JW, Moon SY, Lee JH, et al. Bone marrow analysis of immune cells and apoptosis in patients with systemic lupus erythematosus. *Lupus* 2014;23:975–85.
- [29] Papadaki HA, Boumpas DT, Gibson FM, et al. Increased apoptosis of bone marrow CD34+ cells and impaired function of bone marrow stromal cells in patients with systemic lupus erythematosus. *Br J Haematol* 2001;115:167–74.
- [30] Oka Y, Kameoka J, Hirabayashi Y, et al. Reversible bone marrow dysplasia in patients with systemic lupus erythematosus. *Intern Med* 2008;47:737–42.
- [31] Cao L, Tong H, Xu G, et al. Systemic lupus erythematosus and malignancy risk: a meta-analysis. *PLoS One* 2015;10:e0122964.
- [32] Song L, Wang Y, Zhang J, et al. The risks of cancer development in systemic lupus erythematosus (SLE) patients: a systematic review and meta-analysis. *Arthritis Res Ther* 2018;20:270.
- [33] Bernatsky S, Ramsey-Goldman R, Labrecque J, et al. Cancer risk in systemic lupus: an updated international multi-centre cohort study. *J Autoimmun* 2013;42:130–5.
- [34] Jensen AO, Thomsen HF, Engebjerg MC, et al. Use of oral glucocorticoids and risk of skin cancer and non-Hodgkin lymphoma: a population-based case-control study. *Br J Cancer* 2009;100:200–5.
- [35] Rosenquist R, Davi F, Stamatopoulos K. Antigens in lymphoma development—current knowledge and future directions. *Semin Cancer Biol* 2013;23:397–8.
- [36] Bernatsky S, Joseph L, Boivin JF, et al. The relationship between cancer and medication exposures in systemic lupus erythematosus: a case-cohort study. *Ann Rheum Dis* 2008;67:74–9.
- [37] Dietrich K, Schneid A, Fortuny J, et al. Glucocorticoid therapy and risk of bladder cancer. *Br J Cancer* 2009;101:1316–20.
- [38] Karagas MR, Cushing GL Jr, Greenberg ER, et al. Nonmelanoma skin cancers and glucocorticoid therapy. *Br J Cancer* 2001;85:683–6.
- [39] Wolfe F, Michaud K. Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. *Arthritis Rheum* 2004;50:1740–51.
- [40] Bernatsky S, Clarke AE, Suissa S. Hematologic malignant neoplasms after drug exposure in rheumatoid arthritis. *Arch Intern Med* 2008;168:378–81.
- [41] Anderson LA, Pfeiffer RM, Landgren O, et al. Risks of myeloid malignancies in patients with autoimmune conditions. *Br J Cancer* 2009;100:822–8.
- [42] Grünwald HW, Rosner F. Acute leukemia and immunosuppressive drug use: a review of patients undergoing immunosuppressive therapy for non-neoplastic diseases. *Arch Intern Med* 1979;139:461–6.
- [43] Asten P, Barrett J, Symmons D. Risk of developing certain malignancies is related to duration of immunosuppressive drug exposure in patients with rheumatic diseases. *J Rheumatol* 1999;26:1705–14.
- [44] Kempen JH, Gangaputra S, Daniel E, et al. Long-term risk of malignancy among patients treated with immunosuppressive agents for ocular inflammation: a critical assessment of the evidence. *Am J Ophthalmol* 2008;146: 802-12e1.