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Bone marrow and peripheral blood hematopoietic reserve in patients with B-cell chronic lymphocytic leukemia

We investigated the hematopoietic stem cell compartment of 46 patients with B-cell chronic lymphocytic leukemia (B-CLL). The results indicate that patients had fewer bone marrow stem cells and peripheral blood CFU-GM than did controls. Older patients have fewer committed progenitors in peripheral blood. Stem cells in BM show a more evident inverse relationship with the size of the B-CLL clone.

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Autologous transplantation of bone marrow (BM) and/or peripheral blood (PB) stem cells is an increasingly used treatment for B-CLL,[1] but little is yet known about the residual stem cell compartment and contradictory results have been published.[2-5]

In order to evaluate this hematopoietic reserve, we determined the number of colony-forming units (CFU) and burst-forming units (BFU) of myeloid (GM), erythroid (E) and myelomonocytic progenitors (MM) in BM and PB using short-term *in vitro* culture tests, seeding 10^5 mononuclear cells (MNC) per mL. We analyzed samples from 46 B-CLL patients: 23 males, 23 females, median age 66 years; 16, 19, and 11 patients were in Rai stage 0, I+II, and III+IV, respectively. Four previously treated patients had been untreated for at least 4 months before sampling. BM and PB samples were simultaneously analyzed in 17 patients, BM alone in 25, and PB alone in four patients. The control group was formed of healthy, age and sex-matched individuals: 12 BM donors for allogeneic transplantation and 21 blood donors.

The median count of BM progenitors was lower in B-CLL patients: 11 CFU-GM and 19 BFU-E/10^5 MNC versus 53 CFU-GM and 32 BFU-E/10^5 MNC in controls (p <0.001). The median CFU-GM/10^5 MNC in PB was 3 in B-CLL patients and 21 in controls (p = 0.022). There was no difference in BFU-E counts between B-CLL patients and controls (p = 0.907). The median BM CFU-GEMM count was lower in B-CLL patients than in controls, whereas the difference in PB CFU-GEMM was not statistically significant (Table 1).

Only the number of BM clonogenic cells correlated with evaluated parameters of disease (Table 2), the correlation being positive with preservation of normal hematopoiesis precursors (erythroid and myeloid in BM and $CD34^+$ cells in both BM and PB) and negative with parameters indicating disease progression and tumor mass enlargement (PB leukocyte and lymphocyte counts, proportion of BM lymphocyte infiltration, advanced clinical stage according to the modified Rai classification[6] and total tumor mass score – TTM).[7] The proportion of BM $CD34^+$ cells correlated with PB CFU-GM and BFU-E (p = 0.005). The correlation of lymphocyte proportion in PB leukocytes (%) was negative and significant only with BM CFU-GM.

The BM CFU-GM count was lower in patients with organomegaly (Rai I+II) and BM insufficiency (Rai III+IV) than in Rai stage 0 (8 in Rai I+II and 5 in Rai III+IV vs. 43.5 in Rai 0, p = 0.043). In contrast, BM BFU-E count was lower only in patients with Rai stage III+IV (27 in Rai 0 and 23 in Rai I+II vs. 9.5 in Rai III+IV, p = 0.037). These results suggest a difference between BM CFU-GM and BFU counts in association with clinical stage, the erythroid progenitors being better preserved till more advanced stages. In PB there was no difference in CFU-GM and BFU-E contents according to Rai-stage. TTM was inversely correlated with CFU-GM (r=-0.54, p = 0.026) and BFU-E (r=-60, p = 0.011) in BM. The PB CFU-GM and BFU-E counts were significantly lower in patients over 60 years old (p<0.05 for both).

Reports on the residual stem cell compartment in B-CLL are controversial.[2-5] There are reports of a considerably increased CFU-GM at the time of diagnosis using clonogenic *in vitro* tests,[2]

Table 1. No. of CFU-GM, BFU-E and CFU-GEMM per 10^5 mononuclear cells in bone marrow and peripheral blood samples from patients with B-CLL and from healthy controls.

Cell type	*Source of cells*	*B-CLL Patients Median (10-90% range) N*		*Controls Median (10-90% range) N*		*Statistics p*
CFU-GM	Bone marrow	42	11 (1-58)	12	53 (24-95)	<0.001
	Peripheral blood	21	3 (1-21)	21	21 (9-36)	0.022
BFU-E	Bone marrow	25	19 (6-44)	12	32 (7-47)	<0.001
	Peripheral blood	21	5 (1-42)	21	13 (3-22)	0.907
CFU-GEMM	Bone marrow	24	0 (0-2)	12	6 (0-10)	0.031
	Peripheral blood	21	0 (0-2)	21	3 (0-6)	0.566

Table 2. Hematologic data and correlation coefficients (r, with p – level of correlation significance) of hematologic variables with No. of CFU-GM and BFU-E in bone marrow and peripheral blood in patients with B-CLL.

Parameter	*Median (10-90% range)*		*CFU-GM from*		*BFU-E from*	
			bone marrow	*peripheral blood*	*bone marrow*	*peripheral blood*
In peripheral blood						
Leukocytes	41.3 (11.1-199)	r	-0.447	-0.308	-0.507	-0.366
($\times 10^9$/L)		*p*	0.003	0.173	0.003	0.104
Lymphocytes	34.6 (6.8-179.1)	r	-0.458	-0.352	-0.587	-0.412
($\times 10^9$/L)		*p*	0.002	0.117	0.002	0.064
Lymphocytes in	82.9 (50-94)	r	-0.565	-0.343	-0.332	-0.407
leukocytes rate (%)		*p*	<0.001	0.128	0.105	0.068
$CD34^+$ cells (%)	0.10 (0-0.19)	r	0.278	0.358	0.433	0.416
		p	0.279	0.122	0.094	0.068
In bone marrow						
$CD34^+$ cells (%)	0.25 (0-3.4)	r	0.506	0.660	0.395	0.663
		p	0.016	0.005	0.069	0.005
Lymphocytes (%)	74 (31-99)	r	-0.530	0.033	-0.531	-0.018
		p	<0.001	0.892	0.006	0.939
Erythroid cells (%)	7 (0-23)	r	0.609	0.007	0.447	-0.012
		p	<0.001	0.977	0.025	0.959
Myelomonocytes (%)	17 (1-47)	r	0.528	-0.045	0.523	0.035
		p	<0.001	0.848	0.006	0.885

or an increase in the absolute progenitor cell count in patients with untreated B-CLL.[5] It should, however, be considered that the absolute progenitor cell count need not reflect the residual hematopoietic compartment, given the possible presence of malignant precursors.[8] Other authors reached quite different results, i.e. CFU-GM counts in B-CLL patients that were lower than[3] or similar[4] to those in healthy subjects. We found reduced numbers of PB CFU-GM, especially in older patients. Like us, Sala *et al*.[4] analyzed progenitor cells in BM and found these were less numerous in patients with untreated B-CLL.

The number of BFU-E was almost identical in stable and progressive disease, whereas the number of CFU-GM was higher in Binet stage A.

The finding that BM CFU-GM counts are reduced as early as in Rai I+II clinical stage, and the strong negative correlation between the proportion of lymphocytes in PB leukocytes and BM CFU-GM pointed to possible inhibition by B-CLL lymphocytes.[9] Analyzed disease parameters correlated strongly only with BM committed progenitors. In analogy to different distribution patterns of tumor cells among patients,[10] the distribution of clonogenic hematopoietic stem cells may also vary among patients. This may explain the differences found between the PB and BM compartments in this study and strongly supports the need to analyze both compartments in order to evaluate the residual hematopoietic compartment in B-CLL.

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The importance of follow-up biopsies of clinically suspicious lymphadenopathy in nodular lymphocyte predominant Hodgkin's lymphoma

Although nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) is an indolent disorder, clinically suspicious lymphadenopathy commonly develops during follow-up. In our series of 100 cases of NLPHL, fifteen cases with sequential biopsies were identified. The vast majority showed reactive changes only, emphasizing the importance of histologic sampling of lymphadenopathy in NLPHL patients.

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Nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) is a rare disorder, characterized by a proliferation of isolated large atypical cells embedded in a B-cell rich stromal infiltrate. Lymphadenopathy, a phenomenon that is commonly observed during follow-up in NLPHL patients, may indicate recurrence or signal disease progression to a diffuse large B-cell lymphoma (DLBCL).

A total of 105 NLPHL cases were retrieved from the records of the Department of Pathology of the University Hospitals of the K.U. Leuven. All cases had been diagnosed in the period between September 1990 and December 2001 and consistently followed up by the clinicians of the same institution. In 15 of the 100 confirmed cases of NLPHL, additional biopsies were available (27 biopsies in total), taken either before the diagnosis of NLPHL was established or during the course of the disease.

In fifteen out of 100 patients in whom the diagnosis of NLPHL was confirmed, clinically suspicious enlarged lymph nodes occurred during the course of their disease. Importantly, neither the morphologic nor the immunophenotypic features of the NLPHL observed in these patients were in any respect distinguishable from those in patients who did not develop lymphadenopathy. Four of these 15 patients had a recurrence of NLPHL, and in three patients a DLBCL was found. In ten patients, sequential lymph node biopsies revealed non-specific lymphadenitis with or without progressive transformation of germinal centers.

NLPHL is generally assumed to be an indolent disease, but despite the overall excellent prognosis, disease recurrence has been noted relatively frequently.[1-3] This paradox has been explained by the fact that the relapses typically respond well to treatment. However, important lymphadenopathy occurring in a lymphoma patient is often considered to be a relapse without histopathological confirmation. Indeed, we have found no more than five patients in whom NLPHL relapsed. Only two of these patients experienced multiple relapses. Moreover, several large studies have failed to confirm the classically described pattern of frequent and multiple relapses.[2-3] Hence it is likely that NLPHL is essentially a curable disease in which relapses are only occasionally observed.

Long-term survivors of all subtypes of Hodgkin's lymphomas are at risk of late complications, in particular the developmment of various types of solid tumors, acute leukemia and non-Hodgkin's lymphoma (NHL).[4] Hardly any study on the risk of a second cancer in Hodgkin's lymphoma has been sufficiently large to allow meaningful subgroup analyses with respect to the risk of developing NHL. The few surveys that attempted this analysis demonstrated that the relative risk of NHL was significantly higher for NLPHL patients than for patients with other subtypes of lymphoma, with a reported incidence of 2-10% depending on the stringency of the histology review and the duration of follow-up.[5-7] We found that NLPHL and DLBCL were associated in 3 out of the 100 patients.

Based on a limited number of cases, it has previously been assumed that DLBCL associated with NLPHL has an excellent prognosis, better than that for *de novo* DLBCL.[8] Yet, in all three of our cases with this association, the occurrence of DLBCL precipitated a fulminant downhill clinical course, as reported in several large scale clinical studies.[1-3,5]

Occasionally, the DLBCL arising in a context of NLPHL have been described to show features of T-cell histiocyte-rich B-cell lymphoma (THR-BCL). Based on a number of morphologic and clinical similarities, including the cytological features of the neoplastic cells, their B-cell phenotype, male preponderance, age distribution, and the tendency to transform to a stroma-poor DLBCL, it was proposed that THR-BCL might represent a transformed NLPHL.[9] However, not a single case of NLPHL-associated THR-BCL was identified in our series, underscoring the rarity of histologic progression of NLPHL into THR-BCL.

Our results, like those reported by the European Task Force on Lymphoma,[1] confirm the overall favorable outcome of NLPHL, even in patients who show clinical evidence of recurrence. Yet the good-to-excellent prognosis of the latter patients, which has been ascribed previously to the presumed intrinsically indolent character of relapse, may be explained in part by the fact that clinically suspicious enlarged lymph nodes often show only reactive changes. Occasionally, however, a proliferation that is morphologically recognized as a DLBCL develops on the background of NLPHL. Our findings demonstrate that, contrary to what has been previously suggested, in this context the latter may follow a highly aggressive course, compelling intensive treatment. Hence, apart from its utility in avoiding over-treatment of cases of reactive hyperplasia, biopsies of any clinically suspicious lymphadenopathy is essential in order to prevent under-treatment which may compromise the outcome of cases of NLPHL-associated DLBCL.

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