

## RESEARCH ARTICLE

# Diagnostic and Prognostic Relevance of Bone Marrow Microenvironment Components in Non Hodgkin's Lymphoma Cases Before and After Therapy

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### Abstract

**Objective:** To evaluate stromal cells of the bone marrow microenvironment (BMM) in bone marrow trephine biopsy (BMTB) specimens, with a focus on fibronectin, tumor necrosis factor- alpha (TNF- $\alpha$ ) and L-selectin in Non-Hodgkin's lymphoma (NHL) patients, before and after therapy. **Materials and Methods:** A total of 80 de novo NHL patients, 64 with B-cell lymphomas 80% (follicular cell lymphoma (FCL) in 32, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) in 12, and diffuse large cell lymphoma in 20) and 16 with T-cell lymphomas (20%) all diagnosed as T-Lymphoblastic lymphomas, were evaluated before and after therapy. For comparison, 25 age and sex matched BM donors, were included as a control group. BMTB material and BM aspirates were taken for morphological assessment of stromal cells, the plasma of these samples being examined for TNF $\alpha$  and L-selectin by ELISA, and fibronectin by radial immunodiffusion (RID). **Results:** BM stromal cells comprising reticular macrophages and fibroblasts were elevated in 53.3% of NHL cases at diagnosis, while BM fibronectin levels were decreased and BM TNF $\alpha$  and L-selectin were higher than in controls ( $p < 0.05$ ). In NHL cases, elevated values of BM TNF $\alpha$  and BM L-selectin were associated with signs of aggressive disease, including  $> 1$  extra nodal sites, detectable B symptoms, high grade, BM and CNS invasion, and a high International prognostic index (IPI) ( $p < 0.05$ ). **Conclusion:** BMM components, TNF $\alpha$ , L-selectin and fibronectin, in NHL can be useful in evaluating disease activity, extent and response to treatment and as prognostic markers according to the IPI.

**Keywords:** Bone marrow microenvironment- trephine biopsy- stromal cells- fibronectin- TNF-alpha- L-selectin- NHL

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### Introduction

Accumulating evidence indicates that bone marrow microenvironment (BMM) plays an important role in the pathogenesis of some myeloid and lymphoid hematological malignancies (HM). Functional alterations and Immunophenotypic abnormalities have been described in bone mesenchymal stem cells, obtained from HM patients (Campioni et al., 2014). BMM plays an important role in promoting hematopoietic progenitor cell proliferation and differentiation as well as the controller progress of these developing hematopoietic cells (Greer et al., 2003). The BMM is a complex organization of several cell types including fibroblastic stromal cells, adipocytes, macrophages and endothelial cells. Additional regulatory factors including extracellular matrix (ECM), cytokines, chemokines, and neural peptides are also located in bone marrow (Janowska-Wieczorek et al., 2001). Recent data indicate that, in parallel with leukemogenic events in the hematopoietic system, the niche is converted into an environment with dominant signals favoring cell proliferation and growth, with a combination of these

events (Li and Naves, 2006). Hematopoietic niche refers to a microenvironment, within the specific anatomic location where stem cells are found, which interacts with stem cells to regulate cell fate in vivo or in vitro stem-cell microenvironment (Birbrair and Frenette, 2016).

In order to evaluate the elements of BMM, microscopic examination and serological markers were done to evaluate BMM in the plasma of NHL patients, before therapy and after complete remission, through assessing the BM plasma levels of fibronectin, L-selectin and TNF $\alpha$ , in addition to microscopic examination of BM biopsies to assess the stromal cells and identify their correlation with disease activity, extent and response to treatment as well as their value as prognostic markers in these patients.

### Material and Methods

A total of eighty newly diagnosed NHL patients were enrolled in this study, and followed after treatment until complete remission (CR). Only these 80 NHL patients achieved CR after treatment and follow up. The patients included B-cell lymphoma (B-NHL  $n = 64$ ) (80%), and

T-cell lymphoma (T-NHL n= 16) (20%). B-cell lymphoma patients were divided into follicular cell lymphoma (FCL) (n=32/80)(40%), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) (n=12/80) (15%), diffuse large cell lymphoma (n=20/80)(25%) and T-cell lymphoma patients were all diagnosed as T-cell lymphoblastic lymphoma (n=16/80) (20%). All NHL patients were admitted to the National Cancer Institute, Cairo University (NCI-CU) during the period from February 2012 to May 2015. A Control group was obtained from 25 BM samples of donors of Allogeneic bone marrow transplant (BMT) from BMT Center, Nasser Institute, during the same time.

Clinical characteristics of the studied groups are given in Table (1). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. A written informed consent was obtained from all patients and approval for this study was obtained from Institutional Review Board of the NCI, Cairo University. Inclusion criteria were de novo cases of NHL, diagnosed by standard lab methods, age range 18-78 years, and after therapy, only cases in complete remission (CR).

Criteria of CR were disappearance of all evidence of disease, BM infiltrate cleared on repeated biopsy, and if indeterminate by morphology, immunohistochemistry was negative, spleen or liver were non palpable with disappearance of nodules, according to Cheson et al. (2014). Exclusion criteria for the study were the presence of any inflammatory disease e.g. rheumatoid arthritis, other malignant diseases and previous exposure to chemotherapy, or relapsing patients, and partial or incomplete remission.

All patients were diagnosed according to the standard diagnostic methods including: clinical, lymph node biopsy, cytomorphological, cytochemical, immunological, and cytogenetic evaluation. NHL patients were evaluated according International Prognostic Index (IPI). NHL treatment was according to the standard CHOP protocol, in the form of (C) Cyclophosphamide 750 mg/m<sup>2</sup> IV for 1 day, (H) Doxorubicin (Adriamycin) 50mg/m<sup>2</sup> IV for 1 day, (O) Vincristine (Oncovin) 1-4 mg/m<sup>2</sup> IV for 1 day, (P) Prednisone 100 mg daily for 5 days. This protocol was given once every 3 weeks for 6 doses.

The following methods were done for all NHL patients before receiving any treatment, and after achieving CR, during evaluation and follow up.

1. Bone marrow Aspirate samples were collected for morphology and immunophenotyping, the remaining aspirate was collected in heparinised tubes. After centrifugation at 1,000 xg for 10 min, the plasma was divided into aliquots in sterile tubes and stored at -70°C till use. Immunophenotyping: was done for NHL samples by Flow Cytometry (Larsen, 2000).

2. Bone marrow Trepine biopsy (BMTB) was done to fulfill the diagnosis, staging of NHL and to study the morphology of stromal cells by the standard technique (Williams and Nicholson, 1963).

3. Estimation of serum LDH by the kinetic assay (Handerson, 1995) and serum  $\beta$ 2microglobulin ( $\beta$ 2M) was determined by an immunometric enzyme immunoassay (McCarthy et al., 1994).

4. L-Selectin and TNF $\alpha$  were analyzed in BM plasma by a two-step sandwich ELISA as per the manufacturer's instructions by Diaclone Research (Besançon 25000, France), for in-vitro quantitative determination. Each sample was assayed in duplicate. L-selectin and TNF $\alpha$  were expressed in (ug/ml) and (pg/mL) respectively.

5. Fibronectin was analyzed in BM plasma samples was measured by Radial-Immunodiffusion technique by Mancini et al., (1965). The kit was manufactured by BINDARID, NANORID kits. (The Binding site Ltd., R&D, Birmingham, UK).

#### Response definitions

All responses were defined prospectively. The following definitions were employed according to Cheson et al (2007):

- Complete remission (CR): complete disappearance of all clinical evidence of disease by physical examination, imaging studies, BM aspirate, and/or CSF evaluation, 1 month after completion of last treatment.
- Partial response:  $\geq$ 50% decrease in the sum of the products of the diameters of each measurable lesion, along with the documented presence of residual disease 1 month following completion of the projected course of treatment.
- No response: <50% decrease in the size of measurable lesions, along with the documented presence of residual disease 1 month after completion of treatment.
- Progressive disease: the appearance of new lesions or the enlargement of initial sites of disease during treatment.
- Relapse: histologically proven recurrence of lymphoma/ acute leukemia following the achievement of a complete response (CR).

#### Statistical analysis

Statistical Package for the Social Sciences for Windows (version 12.0, SPSS, Chicago, IL, USA) was used for data management. Chi square and Fisher's exact test were used for testing proportion independence. Data were presented as mean  $\pm$  standard deviation (or median) and frequencies (%). P value is significant when (p< 0.05).

## Results

#### Characteristic and Clinical data of patients and controls

The patients were divided into NHL at diagnosis and after CR, compared to control. The NHL patients were divided into 52/80 (65%) males and 28/80 (35%) females with M:F ratio = 1.8:1, of age range from (18 to 76 years) and median age 55 years. The clinical data of the studied patients are shown in Table (1). B-symptoms, in the form of night sweating, unexplained fever and unexplained loss of more than 10% of the body weight in the preceding 6 months, was present in >50% of patients, weight loss was found in (80%), while fever and night sweat were equally present in (50%) of patients.

Results of hematological laboratory tests and Plasma BM L-selectin, TNF $\alpha$  and FN, serum LDH and B2M, in NHL at diagnosis and after CR are shown in Table (2). Immunophenotypic and pathological classification of patients is clarified in Table (3).

L-Selectin and TNF $\alpha$  plasma BM levels showed

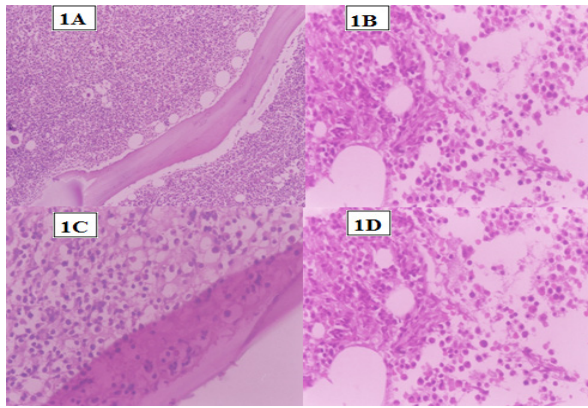


Figure 1 (A, B, C, D). Bone Marrow Trephine Biopsy section of a case of Disseminated Lymphoblastic Lymphoma. Hypercellular trephine BM biopsy showing reduction of erythropoiesis, marked increase in reticular cells, macrophages, moderate increase in fibroblasts and interstitial infiltration by numerous lymphocytes. A case of Disseminated lymphoblastic lymphoma

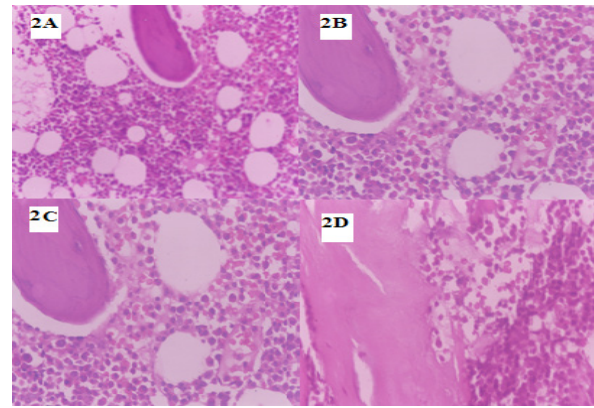


Figure 2 (A, B, C, D). Bone Marrow Trephine Biopsy section of a case of Follicular lymphoma. Normocellular trephine BM biopsy shows normal bone marrow hematopoietic elements and infiltration by small lymphoid cells with few large cells, macrophages, reticular cells, fibroblast and fat cells are increased

significant elevated levels in NHL patients at diagnosis ( $p < 0.001$ ), while after CR, the levels markedly decreased showing statistical significance ( $p < 0.05$ ), ( $p < 0.001$ ) respectively. BM FN mean level was markedly decreased in NHL before therapy ( $p < 0.001$ ). After CR, BM FN was elevated but still low when compared to control group, with no statistical significance ( $p > 0.05$ ).

#### Bone marrow Morphology findings

Bilateral BMTB was performed for all NHL (80 patients) at diagnosis and after CR for evaluation. At diagnosis, BM microscopic findings showed hypercellularity in 36/80 (45%), and normocellularity in 44/80 (55%) of patients, reduced erythropoiesis in 24/80 (30%), granulopoiesis in 28/80 (35%), and decreased megakaryopoiesis in 24/80 (30%).

Evaluation of BM stromal cells revealed increased BM reticular cells, macrophages and fibroblasts in 44/80 (55%) with variable degrees, with reduced adipocytes in 20/80 (25%), and elevated adipocytes in only 6/80 (7.5%) and normal in the remaining 54/80 (67.5%) patients. BM fibrosis was seen in 8/80 (10%) with mild and marked fibrosis. BM infiltration was seen in 60/80 (75%) of NHL patients at diagnosis, distributed into 16/16 (100%) of lymphoblastic lymphoma and 12/12 (100%) of CLL, in 20/32 (62.5%) of follicular lymphoma and 12/20 (60%) of diffuse large cell lymphoma.

Diffuse pattern of infiltration in BM biopsy specimens was present in 54/80 (67.5%) patients, paratrabecular infiltration in 20/80 (25%) and focal infiltration in 6/80 (7.5%) patients.

After CR, microscopic findings improved to normocellular BM in 90% and hypocellular BM in 10% of patients, normal erythropoiesis, granulopoiesis and megakaryopoiesis in all patients except for 6/80 (7.5%) patients with reduced granulopoiesis and 12/80 (15%) patients with reduced megakaryocytes.

Evaluation of BM stromal cells showed increased reticular cells and macrophages in 20/80 (25%), while fibroblasts were increased in 12/80 (15%) of patients.

#### Correlation Studies in NHL at diagnosis

The correlation matrix, in (Table 6) showed positive correlations between BM TNF $\alpha$  and TLC ( $r = 0.587$ ,  $p < 0.001$ ), % blast cells in peripheral blood (PB) ( $r = 0.759$ ,  $p < 0.001$ ), % blast cells in B.M ( $r = 0.939$ ,  $p < 0.001$ ) and IPI, ( $r = 0.677$ ,  $p < 0.001$ ) in contrast to a significant negative correlation between BM TNF $\alpha$ , and HB level ( $r = -0.774$ ,  $p < 0.001$ ).

While a negative correlation was found between with L-selectin and HB level in NHL at diagnosis ( $r = -0.855$ ,  $p < 0.001$ ), TLC ( $r = 0.699$ ,  $p < 0.001$ ), % blast cells in PB ( $r = 0.581$ ,  $p < 0.001$ ), % blast in B.M ( $r = 0.684$ ,  $p < 0.001$ ) and IPI ( $r = 0.661$ ,  $p < 0.001$ ).

While there was a significant positive correlation between BM FN and HB level ( $r = 0.553$ ,  $p < 0.05$ ), with insignificant correlation between BM fibronectin, and TLC ( $r = -0.188$ ,  $p = 0.427$ )

#### Association Of BMM Analytes With Signs Of Disease Activity, Extent And Prognosis In NHL

We studied the association of BM analytes with signs of disease activity, extent and prognosis as shown in Table (5). The signs included BM invasion, CNS invasion, B-symptoms. The upper limit (UL) of BM TNF $\alpha$  in the control group was 50 pg/mL, UL of L-selectin, LDH and  $\beta$ 2M was (8ug/ml), (380 U/L), (1.8 U/L) respectively. Any levels above this value were considered pathologically high. Patients were evaluated according to the UL and correlated to signs of disease activity, extent and prognosis.

Elevated BM L-selectin value ( $> 8\text{ug/ml}$ ) and BM TNF $\alpha$  value ( $> 50\text{ pg/ml}$ ), serum LDH ( $> 380\text{ U/L}$ ), and  $\beta$ 2M ( $> 1.8\text{ U/L}$ ) were associated with BM invasion in (80%) of patients, with a statistical significance ( $p < 0.05$ ), with CNS invasion in 12/80 (15%) and with B symptoms in 40/80 (50%) of patients, with a non statistical significance ( $p > 0.05$ ).

A comparative study was done between signs of disease activity in NHL at diagnosis and after CR (Table 6), in association with mean values of BMM analytes.

Elevated BM TNF and BM L-Selectin mean values, were higher in NHL patients at diagnosis, with



Table 1. Clinical Characteristics of NHL at Diagnosis and Control

Parameter	Group I NHL at diagnosis			Control Group	
	Data	N	%	N	%
Number of Patients		80	100	25	100
Gender	Male: Female	52 M :28 F	1.8:1	20 M :5 F	4:1
Age range (years)		18-76		29-38	
Median Age (years)		55		34	
Immunophenotyping	B-NHL	64	80%		
	T-NHL	16	20%		
Anemia	HB levels < 12gm/ml	56	70%		
Leucocytosis	TLC > 11x10 <sup>3</sup> U/L	28	35%		
Thrombocytopenia.	Platelets < 150x10 <sup>9</sup> U/L	32	40%		
Weight Loss	Loss >10% of Body Wt in 6mths	64	80%		
Fever	Body temperature > 37°C	40	50%		
CNS invasion	> 1% Blasts in CNS	12	15%		
Bone marrow infiltration	> 5% blasts in BM	60	75%		
Splenomegaly		72	90%		
Hepatomegaly		60	75%		
Lymphadenopathy		48	60%		
Extranodal Sites	<1	60	75%		
	>1	20	25%		
	BM	60	75%		
	CNS	8	10%		
	GIT	8	10%		
	Liver	4	5%		
Ann Arbor Stages	1	0	0%		
	2	4	5%		
	3	12	15%		
	4	64	80%		
B-symptoms	No	40	50%		
	Yes	40	50%		
International prognostic index	Low risk 0-1	8	10%		
	Low intermediate 2	12	15%		
	High intermediate 3	36	45%		
	High risk 4-5	24	30%		

B-cell-symptoms, in comparison to patients without B-symptoms, ( $p < 0.05$ ) and ( $p > 0.05$ ), respectively. After CR, it dropped in both groups of patients ( $p < 0.05$ ) and ( $p > 0.05$ ).

Elevated BM TNF $\alpha$  and L-selectin mean values were significantly higher in NHL patients with extra nodal sites  $> 1$ , than with extra nodal sites  $\leq 1$  at diagnosis, and dropped significantly after CR, ( $P < 0.001$ ). BM TNF $\alpha$  and BM L-selectin mean values were elevated in high-grade lymphoma in comparison to low/intermediate grade lymphoma at diagnosis, and dropped after CR, BM TNF ( $P > 0.05$ ), and statistically significant in BM L-selectin ( $P < 0.05$ ).

Elevated BM TNF  $\alpha$  and BM L-Selectin mean values were significantly associated with T-cell Lymphoma and high IPI at diagnosis, than in CR, when compared to B-cell Lymphoma and other grades of IPI (Low, intermediate and

Intermediate high), ( $P < 0.05$ ). Decreased BM L-selectin in CR showed no statistical significance between T-cell and B-cell NHL and between the different scores of IPI ( $p > 0.05$ ).

Decreased BM FN mean values were markedly lowered in NHL patients at diagnosis with B-symptoms, than without B-symptoms ( $p > 0.05$ ), while a statistically significant increase was found in CR in both groups of patients ( $p < 0.001$ ). Decreased BM FN mean values were lower in patients with extra nodal site  $> 1$  than those patients with extra nodal sites  $\leq 1$  and T-cell NHL in comparison to B-cell NHL, at diagnosis and increased significantly after CR ( $P < 0.001$ ) and ( $p < 0.05$ )

BM FN was insignificantly decreased in patients with high-grade lymphoma when compared to low/intermediate grade lymphoma at diagnosis and after CR ( $p$  value  $> 0.05$ ). Decreased BM FN was of no significance

Table 2. Hematological Findings and BMM Analytes of NHL Patients at Diagnosis and After CR

Parameter	NHL	NHL	Control	P value
	At Diagnosis	After CR		
	Mean±SD	Mean±SD	Mean±SD	
N. of Patients	80	80	25	
Age	43.2±17.3		35.2±2.6	p> 0.05
Hb (g/dl)	10.8±2.3	13.4±1.2	14.3±0.8	P<0.001*
TLC (x103/l)	19.3±18.9	6.4±2.0	6.5±1.9	P=0.006*
Platelets(x109/l)	182.0±90.3	207.0±87.5	265.1±54.2	P=0.240
PB blasts%	3.5±6.5	-	-	-
BM blasts%	9.4±18.1	-	-	P=0.008*
Bone Marrow Microenvironment Analytes				
L-Selectin ug/mL	21.2±10.7	6.8±5.2	4.1±1.5	p<0.001*
TNF-α pg/mL	134.7±121.5	46.4±28.4	42.1±10.8	p< 0.001*
Fibronectin mg/L	79.1±21.0	136.5±35.5	164.0±57.4	p< 0.001*
LDH (ul/L)	570.0±161.8	282.8±66.4	278.0±51.2	p< 0.001*
B <sub>2</sub> Microglobulin (mg/L)	2.9±0.7	1.3±0.3	1.3±0.4	p< 0.001*

\*P&lt; 0.05 significant

with different IPI grades (P>0.05).

Elevated serum LDH mean value was detected in NHL patients at diagnosis with extra nodal sites >1 and T-cell NHL in comparison to B-cell NHL and high IPI index and decreased significantly after CR (P<0.001). Increased serum β2M mean value was significant in high IPI index at diagnosis and after CR (p<0.05), and in patients with extra nodal sites >1 in CR (p<0.050).

## Discussion

The study of BMM in NHL shows how the malignant process can cause a significant disturbance in the equilibrium of this microenvironment. This work was conducted to study the BMM elements in NHL patients before and after therapy, in CR, to evaluate the impact of chemotherapy on these patients. For this purpose, we estimated BM TNFα, BM L-selectin and Fibronectin in 80 NHL at diagnosis and after achieving CR, with 25 donors for Allogeneic BMT as control group. BM aspiration and trephine biopsy were performed for all patients before therapy and after CR for evaluation of BM stromal cells, also to examine infiltration of BM, its types, and degrees.

BM levels of L-selectin in NHL patients at diagnosis were found to be higher than control group (P< 0.05), and decreased significantly at CR (P<0.05), but still higher

than 5 folds of control group (P<0.05). Yaris et al., (2001), reported that L-selectin was increased in 63% of patients and was positively correlated with disseminated disease.

Plasma BM TNFα was above the upper limit in 90% of NHL patients before therapy and decreased markedly at CR, (P<0.05), but still higher than control group (P<0.05). In agreement with our results, Baseggio et al., (2001), stated that serum TNFα level was increased in NHL patients before treatment and returned to near normal level in CR. In the present study, elevated plasma BM TNFα level, was associated with B-symptoms (P<0.05), in agreement with Metkar et al., (2000), and was associated with bad prognosis.

We found that BM TNFα was more elevated in patients with extra nodal sites >1 and related to BM involvement at diagnosis. Patients with BM TNFα >50 pg/ml were associated with 1.5 fold higher frequency of BM invasion at diagnosis. This is in agreement with Seki et al., (2002), who demonstrated that high serum TNFα level was associated with increased incidence of bone marrow invasion.

Plasma BM TNFα was elevated in high grade lymphoma when compared to low/intermediate grade lymphoma (P> 0.05), and in cases with high IPI in comparison with intermediate/high, intermediate/low and low IPI patients at diagnosis (p<0.05). This was agreed

Table 3. Immunophenotypic and Pathological Classification of NHL

Item	NHL N=80	
	B-cell NHL N= 64 (80%)	T- cell NHL N= 16 (20%)
Immunophenotype	CD19+ve, CD20+ve	CD3+ve, CD4+ve or CD8+ve
Pathology	Diffuse large cell lymphoma 20 (25%)	Malignant lymphoblastic lymphoma 16 (20%)
	Small lymphocytic lymphoma /CLL 12(15%)	-
	Follicular lymphoma 32 (40%)	-
Total	64 (80%)	16 (20%)

CD, Cluster of Differentiation ; CLL, Chronic Lymphocytic Leukemia

Table 4. Correlation Matrix of All Studied Parameters and Hematological Parameters in NHL at Diagnosis

Variables	LDH		Fibro nectin		TNF alpha		L- selectin B <sub>2</sub>		microglobulin	
	r**	p	r	p	r	p	r	p	r	p
Fibronectin	-0.435	0.055								
TNF alpha	0.799	0.001*	-0.507	0.023*						
L-selectin	0.713	0.001*	-0.479	0.033*	0.755	0.001*				
B2- microglobulin	0.654	0.002*	-0.297	0.204	0.454	0.044*	0.571	0.008*		
Hb	-0.714	0.001*	0.553	0.011*	-0.774	0.001*	-0.855	0.001*	-0.772	0.001*
T-leucocytic count	0.516	0.020*	-0.188	0.427	0.587	0.006*	0.699	0.001*		
% Blast Cells in PB	0.715	0.001*	-0.665	0.001*	0.759	0.001*	0.581	0.007*	0.485	0.007*
% Blast cells in B.M	0.671	0.001	-0.518	0.019	0.939	0.001	0.648	0.002	0.239	0.31
IPI	0.523	0.018*	-0.662	0.001*	0.677	0.001*	0.661	0.002*	0.593	0.006*

\*P < 0.05 significant ; \*\* r, pearson correlation; IPI, International Prognostic Index

with Ohshima et al., (2000), who found that serum TNF $\alpha$  level was increased in aggressive type of lymphoma more than patients with lower grade lymphoma. There were positive correlations between BM TNF $\alpha$  and serum LDH,  $\alpha$ 2M, TLC and % of blast cells in PB, and negative correlations between BM TNF $\alpha$  and HB level. This is in agreement with Luis et al., (2001), who found that a high serum TNF $\alpha$  level was correlated with unfavorable features of the disease as high serum LDH and high serum  $\alpha$ <sub>2</sub>M.

BM FN levels, before therapy in NHL, were found to be lower than the upper limit (UL) of control group, in 100% of patients, and increased markedly after remission (P<0.05). The decreased plasma BM FN levels were associated with B-symptoms and BM involvement, in high grade lymphoma, and with extra nodal sites > 1 and NHL with high IPI (P<0.05). The decrease in FN levels and return to normal level after remission, may be due to increased consumption of FN in the expanded mononuclear phagocytic system presenting in liver and spleen, and also to reduced hepatic synthesis (Brenner et al., 2000).

Serum LDH and  $\alpha$ 2M were significantly increased in NHL patients at diagnosis (before treatment), (p<0.05), and decreased to near normal levels after remission. Elevated serum LDH and  $\beta$ 2M showed association with BM invasion and B-cell symptoms in NHL.

In NHL, the dynamic reciprocal interaction between the BMM and the malignant hematological cells continues throughout disease progression, and is related to environment-mediated drug resistance (Meads et al., 2008).

In our study, microscopic examination of BM trephine biopsies of NHL patients, at diagnosis revealed that bone marrow stromal cells were abundant at diagnosis. After CR, there was an increase in 20% of patients in reticular cells and macrophages, and 15%. The overall incidence of marrow invasion at diagnosis was 75%, with the higher incidence of diffuse marrow infiltration that was detected in 66.7% of patients, followed by para-trabecular, focal and random infiltration that were seen in 26.6% and 6.7% of patients, respectively. Our results are in agreement with (Lim and Peh 2000) who reported that diffuse infiltration was seen in 71.4% followed by para-trabecular in 19%

Table 5. Association of BM Invasion, CNS Invasion, B-Symptoms with Studied Parameters in NHL Before Treatment

Biochemical parameters/ Clinical data	TNF- $\alpha$	L-selectin	LDH	$\beta$ <sub>2</sub> microglobulin
	>50pg/ml*	>8 ug/ml*	>380 U/L*	>1.8 U/L*
	no (%)	no (%)	no (%)	no (%)
BM invasion				
Negative	20 (25%)	20 (25%)	20 (25%)	22 (27.5%)
Positive	60 (75%)	60 (75%)	60 (75%)	58 (72.5%)
P value	0.050**	0.050**	0.050**	0.050**
CNS invasion				
Negative	68 (85%)	70(87.5%)	68(85%)	74 (92.5%)
Positive	12 (15%)	10 (12.5%)	12 (15%)	6 (7.5%)
P value	1	0.984	1	0.995
B-symptoms				
Negative	40(50%)	40(50%)	40(50%)	40(50%)
Positive	40(50%)	40(50%)	40(50%)	40(50%)
P value	0.474	0.474	0.474	0.474

\*\* P value < 0.05 is significant

Table 6. Comparative Study between Signs of Disease Activity in NHL At Diagnosis and After CR Regarding the Studied Parameters

Signs	No. (%)	TNF- $\alpha$		L-selectin		Fibronectin		LDH		B2 microglobulin	
		At Diagnosis	After CR	At Diagnosis	After CR	At Diagnosis	After CR	At Diagnosis	After CR	At Diagnosis	After CR
Without B-symptoms	40/80(50%)	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD	Mean+SD
		76.9+25.4	32.7+10.5	16.90+7.23	5.43+2.25	92.00+17.51	159.50+28.91	508.60+115.02	256.1+47.4	2.8+0.8	1.3+0.3
With B-symptoms	40/80(50%)	192.6+152.1	60.1+34.2	25.55+12.18	8.21+6.88	66.30+16.17	113.50+25.60	631.50+183.51	309.5+174.0	3.0+0.7	1.4+0.4
p value		0.040*	0.035*	0.073	0.241	0.366	0.001**	0.093	0.07	0.692	0.4
Extra Nodal site < 1	60/80(75%)	81.3+35.3	33.7+89.2	16.37+6.4	5.16+2.8	86.13+19.66	149+99	511.1+113.1	256.1+39.9	2.8+0.7	1.3+0.3
Extra Nodal site >1	20/80(25%)	295+150.9	89.2+36.27	35.8+6.83	11.8+8.4	58.2+4.49	99+22.75	746+167.4	363.0+68.0	3.3+0.6	1.6+0.3
p value		0.001**	0.001**	0.001**	0.001**	0.001**	0.003**	0.002*	0.001**	0.202	0.05
Low/ Intermediate Grade Lymphoma	60/80(75%)	117.9+124.2	43.6+22	16.2+7.8	6.1+1.7	86+21.33	154+23.29	532.4+151.6	263.5+54.8	2.8+0.7	1.3+0.3
High Grade Lymphoma	20/80(25%)	200.1+145.4	59.7+20.7	31.4+13.3	8.3+1.6	83.09+21.57	140+38.54	640+176.9	318.6+75.1	3.2+0.6	1.5+0.3
(p value)		0.199	0.129	0.023*	0.012*	0.806	0.469	0.162	0.076	0.251	0.24
B-cell- Lymphoma	64/80(80%)	108.13+112.7	38.25+21.3	17.84+8.57	5.59+2.64	84+20.82	144.4+34.35	532.56+138.2	263.8+49.5	2.8+0.69	1.3+0.3
T-cell- Lymphoma	16/80(20%)	241.2+105.3	79+32.57	34.75+7.41	11.75+9.74	59.75+0.3	105+21.21	720+181.29	358.7+77.7	3.33+0.7	1.5+0.4
p value		0.047*	0.006**	0.002**	0.296	0.001**	0.044*	0.034*	0.006**	0.19	0.19
IPI prognostic Index											
Low 0-1	8/80(10%)	40.3+4.5	20.3+0.6	10+6.1	5+2.2	100+28.28	152.5+45.96	406.7+64.3	288.3+22.5	1.9+0.4	1+0.2
Low	12/80(15%)	78.8+18.0	40.8+7.8	14+4.8	5.8+2	98.33+24.66	160+30	522.5+152.2	252.8+72.6	2.7+0.6	1.3+0.3
Intermediate 2											
High	36/80(45%)	106.9+34.1	48+2.5	18.3+4.5	7.1+0.7	76.89+13.4	141.1+29.02	553.6+95.7	267.9+18.0	3.1+0.6	1.3+0.3
Intermediate 4											
High risk 4-5	24/80(30%)	291+170.3	2.5+27.4	36.2+10.9	8.2+2.1	66+19.52	112.5+38.83	702.7+183.6	347.5+71.7	3.3+0.6	1.6+0.3
p value		0.004**	0.002**	0.001**	0.066	0.059	0.206	0.041*	0.015*	0.016*	0.033*

CR, Complete Remission; IPI, International Prognostic Index; \*P &lt; 0.05 is significant; \*\* P &lt; 0.01 is highly significant

and focal infiltration in 4.8% of NHL, and they reported that marrow lymphocytosis was more common in T-NHL than B-NHL patients at diagnosis (40% versus 13.9%) with no statistically significant difference.

This study proves that the different components of BMM are greatly affected by malignant transformation of the hematopoietic cells in NHL. Elevated BM plasma TNF $\alpha$ , and L-selectin were associated with B-symptoms, BM involvement, high grade lymphoma, extranodal sites > 1, high IPI at diagnosis than in CR. BM plasma fibronectin was significantly decreased in NHL patients at diagnosis unlike in CR, in addition to increased numbers of BM stromal cells at diagnosis, in contrast to CR.

In conclusion, Fibronectin, L-selectin and TNF $\alpha$  can be used as markers for the disturbance of the hematopoietic microenvironment. Also, bone marrow trephine biopsies may be used as a tool to assess the BMM in NHL for diagnosis and follow up. The studied parameters can be used as markers for early relapse as well as remission after treatment. After clarifying the role of the BM stromal cells in NHL, future studies can provide novel therapies that target cells of BM microenvironment.

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