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# Chapter 4

## **Primary Lymphoma of Bone: Extranodal Lymphoma with Favourable Survival Independent of Germinal Centre, Post Germinal Centre, or Indeterminate Phenotype**

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## Abstract

**Aims:** To determine prognostic significance of immunohistochemical markers and investigate possible germinal centre (GC) derivation in primary lymphoma of bone (PLB).

**Methods:** In this study we have investigated the immunohistochemical expression of BCL-6, CD10, BCL-2, p53, CD30 and CD44 and MUM-1 in thirty-six patients with PLB. All cases were clinically staged and cases of secondary bone involvement of primary nodal lymphomas were excluded, prior to immunostaining. Clinical charts were reviewed for clinical symptoms and therapy given and survival post-biopsy was calculated.

**Results:** All patients presented with pain and a palpable mass. The majority showed centroblastic-multilobated morphology, half of the cases (19/36) displayed a GC phenotype (CD10+BCL-6+ or CD10-BCL-6+MUM-1-), whereas 8/36 cases demonstrated a non-GC phenotype (CD10-BCL-6- or CD10-BCL-6+ MUM-1+). Nine cases were of indeterminate phenotype (CD10-BCL-6+; MUM-1 not available). Eight out of 22 evaluated patient samples showed immunoreactivity for MUM-1. Most patients (31/36) received combination therapy in the form of polychemotherapy and radiotherapy. The 5-year overall survival was 75%. No significant difference in survival was found between the three different tumour phenotypes, or for the tested antigens individually. Age at presentation and stage of disease were of significant influence on 5-year overall survival. Survival rates were 90% for the patients under 60 years of age and 40% for those of 60 years and older. Furthermore, survival rates were 90% for stage I vs. 41% for stage IV, respectively.

**Conclusion:** This study illustrates the homogeneity of PLB. The majority of cases is of the GC phenotype and has a favourable prognosis.

## Introduction

Primary non-Hodgkin lymphoma of bone (PLB) is a rare neoplastic disorder, comprising 5% of extranodal lymphomas and less than 1% of all non-Hodgkin lymphomas.[1] It is an extranodal subtype of mostly diffuse large B cell lymphoma (DLBCL), which is a heterogeneous group of lymphomas.

Studies on extranodal lymphomas are rare, even though the incidence of extranodal lymphoma in Western countries has increased in the last 40 years.

Immunohistochemical studies on PLB are even rarer, likely because of the limited availability of tissue specimens of this disorder and technological difficulties in handling osseous tumour material.

PLB has morphological homogeneity and a relatively favourable clinical behaviour with a 5-year overall survival of 90% for stage I disease, as published in our previous report.[2,3]

In the last decade, insight into B-cell development and lymphoma pathogenesis has increased substantially due to the increasing availability of well-defined histogenetic markers. These include the expression of BCL-6 and CD10 protein, which are considered germinal centre (GC) markers, and of MUM1/IRF4, which denotes the final step of intra-GC B-cell differentiation, as well as subsequent steps of B-cell maturation towards plasma cells.[4] The expression of the anti-apoptotic factor BCL-2 and of the surface adhesion molecule CD44 is normally down-regulated in GC B-cells.[5,6] Moreover, genotypic analyses have revealed several structural and numerical chromosomal changes in DLBCL, including translocations t(14;18)(q32;q21) and t(3;14)(q27;q32) that involve BCL-2 and BCL-6 genes, respectively. In numerous studies on DLBCL the effect of these chromosomal changes on prognosis has been debated, but a final conclusion cannot be drawn.[7-9]

Research in gene expression profiling has led to the concept that most DLBCL derive from GC B-cells or from their descendants, i.e. activated B-cells or non-GC B-cells.[10] Since most PLB have a centroblastic phenotype, it has been postulated that PLB represents a de novo DLBCL with a genetic relationship to GC B-cells.[11]

Recent studies have analyzed the expression of GC B-cell-associated antigens and other markers in PLB, showing that at least part of the patients with PLB have a GC signature, which was associated with a favourable clinical outcome.[7,11,12]

In the current study, immunohistochemical staining for BCL-6, CD10, BCL-2, MUM-1, p53, CD30 and CD44 was performed in 36 PLB patients to determine both possible GC origin of these lymphomas as well as the prognostic significance of these markers.

## Material and Methods

### Patient Selection

In this study we included the previously described cohort of patients combined with newly registered cases from 1995 until 2001.[2] This new cohort consisted of 83 cases. PLB was defined as a histologically proven non-Hodgkin lymphoma arising within the medullary cavity of a bone, with or without regional lymph node involvement, but without evidence of other extranodal involvement. Multiple bone lesions were acceptable as long as there was no evidence of earlier lymphoma involvement elsewhere. All cases selected were clinicopathologically reviewed independently by two pathologists, if needed with application of additional immunohistochemical stainings to confirm the diagnosis. Of the originally identified group of 106 patients 30 patients were excluded because of a different diagnosis after review of the histological slides, combined with new immunohistochemical data or clinical follow-up as described in our previous study. Clinical follow-up was updated. Adequate paraffin material for additional immunohistochemical studies was available in 36 cases, of which 14 cases were not described in our previous study.

### Histological Classification and Immunohistochemistry (table 1)

The pathological diagnosis was established according to the WHO classification [13] using standard histological criteria and immunohistochemistry using antibodies directed against Vimentin, CD45, CD3, CD20, CD79a, and CD99. Immunohistochemical staining was performed on 4  $\mu$ m sections of formalin-fixed, paraffin-embedded tissues, using standard procedures as detailed elsewhere.[2] In addition to the diagnostic marker panel listed above a set of markers relevant to a GC/non-GC phenotype or relevant to survival was used. The details of the antibodies are given in table 1.

### Immunohistochemical Scoring

Two pathologists assessed all cases independently. Three categories were defined: negative, 30-75% positivity or >75% positivity, reflecting the percentage of positive tumour cells.

### Statistical Methods

Survival curves were plotted following the Kaplan-Meier method and tested for statistical significance using the log-rank test. Overall survival (OS) was calculated from the date of diagnosis until death (all causes) or last-follow-up. The multivariate Wilson regression test was used to determine statistic significance of the parameters independent of the known IPI risk factors age and tumour stage.

**Table 1.** Antibodies Used for Immunohistochemistry

Marker	Clone	Producer	Type	Isotype
BCL-2	124	Dako	Mouse	IgG1
BCL-6	PG-B6P	Dako	Mouse	IgG-k
CD-3		Dako	Rabbit	Polyclonal
CD10	56C6	Neomarkers	Mouse	IgG1
CD20	L26	Dako	Mouse	IgG2a-k
CD30	BER-H2	Dako	Mouse	IgG1
CD44	156-3C11	Neomarkers	Mouse	IgG1-k
CD45	CLA	Neomarkers	Mouse	IgG1-k
P53	DO-7	Neomarkers	Mouse	IgG2b-k
CD79a	JCB117	Dako	Mouse	IgG1-k
CD99	O13	Zymed	Mouse	IgG1
MUM-1	MUM-1p	Dako	Mouse	IgG1

## Results

### Clinical Features (table 2)

A group of 36 patients with PLB was studied. All the patients presented with pain and/or a palpable mass, most often at a single location in one of the long bones.

Multifocal bone involvement (scored as stage IV) was noted in 10 cases. Twelve out of all 36 patients had undergone an incomplete staging procedure, according to the present standards. All stage IV patients had more than one bone localization and none had iliac crest bone marrow involvement.

The male:female ratio was 26:10. The mean age at presentation was 48 years, ranging from 18 years to 79 years. Clinical findings, including age, gender, primary location of tumour, treatment and outcome are summarized in table 2.

The IPI index could be calculated in 26 of the 36 cases. If unknown, this was mostly due to lacking laboratory results. The mean score was 2. The 5-year overall survival of this cohort was 75%. Most patients (31) received combination therapy in the form of poly-chemotherapy or surgical resection with radiotherapy. All chemotherapy courses were given with curative intentions. It consisted of cyclophosphamide, doxorubicin, vincristin, prednisone (CHOP) (16x), or CHOP-like: cyclophosphamide, teniposide, doxorubicin, vincristin, bleomycin, prednisone (CHVmP/BV) (8x) and prednisone, methotrexate, doxorubicin, cyclophosphamide, epipodophyllotoxin VP-16 (PROMACE) (5x).

Twenty-nine patients (80%) demonstrated a complete remission, including one spontaneous remission. The latter was reviewed on multiple occasions by different pathologists and haematologists, with the original diagnosis confirmed each time. To our knowledge this is the first case of PLB with a spontaneous remission mentioned in a study. Follow-up was available in all but one case. The median follow-up was 79 months. Four patients had progressive disease and died of disease. One patient had no change of disease and died during therapy.

**Table 2.** Clinical Characteristics of the Patient Population Studied

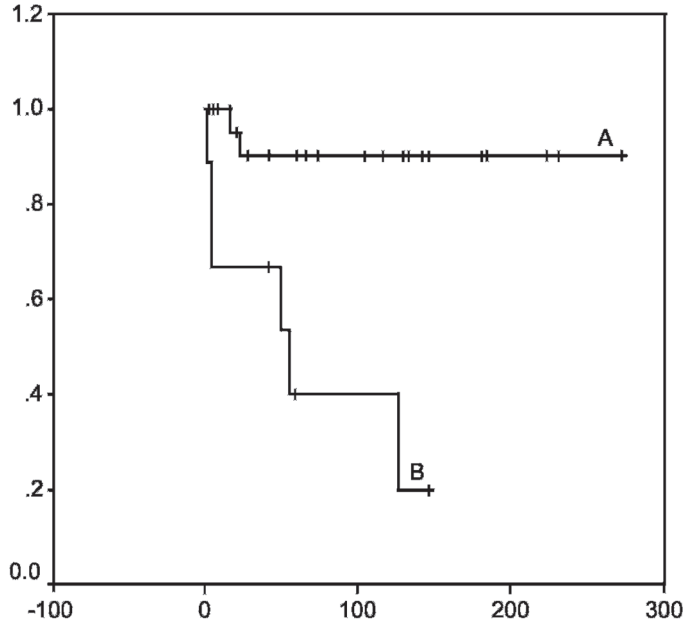
Patient	Sex	Age	Primary Location	Multifocal Disease	Therapy	Result	Relapse
1	M	59	Clavicle		RT + SUR	4	
2	F	61	Humerus		RT + SUR	4	
3	M	50	Femur		RT + SUR	1	
4	M	59	Femur	IV	RT + SUR	4	
5	F	53	Femur		RT + SUR + CHOP-like	1	
6	F	41	Femur		RT + CHOP	1	
7	M	54	Scapula + Humerus		RT + SUR + CHOP-like	1	
8	M	23	Shoulder		RT + CHOP-like	1	
9	M	28	Clavicle		RT + CHOP	1	
10	M	69	Femur		RT + SUR + CHOP	1	
11	M	18	Vertebral Column		RT + SUR + CHOP-like	1	
12	M	30	Femur	IV	Unknown	9	
13	M	65	Humerus		RT + CHOP-like	1	
14	F	38	Hipjoint	IV	None	1	
15	M	40	Femur		CHOP	1	
16	M	20	Femur		RT + CHOP	1	
17	M	23	Femur		RT + SUR	1	
18	F	18	Femur		RT + CHOP	1	
19	M	61	Os Pelvis		RT + SUR + CHOP-like	9	
20	M	30	Femur	IV	RT + CHOP	1	28 months
21	F	23	Scapula		RT + SUR + CHOP-like	1	
22	M	31	Femur		RT + CHOP-like	1	
23	M	72	Humerus	IV	RT + CHOP	1	
24	F	51	Vertebral Column	IV	RT + CHOP	1	
25	M	58	Humerus		RT + CHOP-like	1	
26	F	52	Os Pubis		RT + CHOP-like	1	
27	M	27	Femur		RT + CHOP-like	1	
28	M	79	Humerus	IV	RT + SUR + CHOP	4	
29	M	46	Femur		RT + SUR + CHOP	1	
30	M	51	Hipjoint		CHOP	1	
31	F	78	Femur	IV	CHOP	1	
32	F	71	Femur	IV	RT + SUR + CHOP	1	
33	M	29	Humerus		RT + CHOP	1	
34	M	72	Os Ilium		RT + CHOP-like	1	
35	M	75	Femur	IV	SUR + CHOP	3	
36	M	46	Humerus		RT + CHOP-like	1	

1 Ann Arbor stage; Stage IV is Multifocal

2 RT: radiotherapy; SUR: surgery; CHOP and CHOP-like: chemotherapy

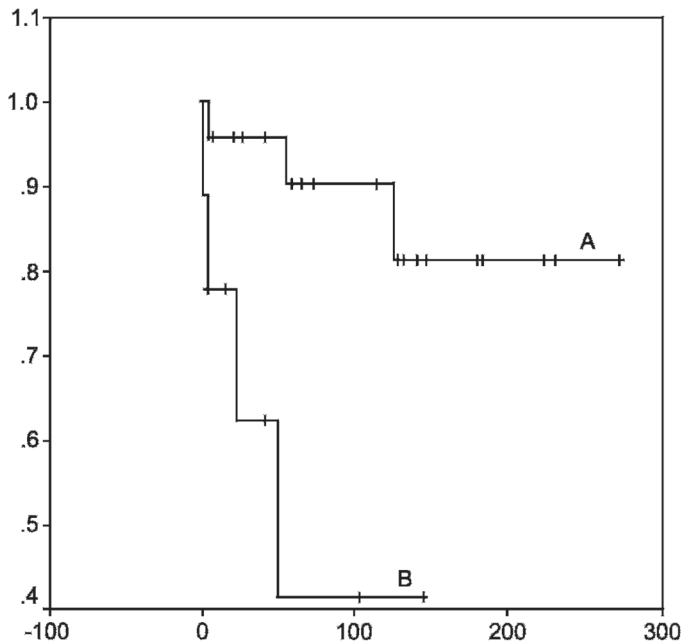
3 1: complete remission; 3: no change; 4: progressive disease; 9: no evaluation possible

**Figure 1.** Kaplan-Meier overall survival curve of age at diagnosis ( $p=0.0004$ )  
**A:** survival % (in months) of patients of 60 years and younger  
**B:** survival % (in months) of patients above 60 years of age



One patient experienced a nodal relapse at 28 months, with complete remission after second-line chemotherapy and no evidence of disease at 118 months.

There was a significant association between the IPI index factors age and stage of disease and survival. The overall 5-year survival for the patients under 60 years at the time of diagnosis



**Figure 2.** Kaplan-Meier overall survival curve of stage of disease ( $p=0.0085$ )  
**A:** survival % (in months) of stage I patients  
**B:** survival % (in months) of stage IV patients



**Table 3.** Selection of Histological and Immunohistochemical Features

Patient	Morphology	CD10	BCL-6	MUM-1	Phenotype	BCL-2	P53	CD30	CD44
1	Centro-multi	-	-	-	Non-GC	-	-	-	++
2	Diff centro	-	+	+	Non-GC	++	++	-	++
3	Diff centro	+	+		GC	-	+	-	++
4	Diff centro	-	+		Indet	+	-	-	++
5	Diff centro	++	++		GC	-	-	-	-
6	Centro-multi	++	+	-	GC	-	-	-	+
7	Diff centro	-	+		Indet	++	-	-	++
8	Centro-multi	-	+		Indet	-		++	-
9	Centro-multi	-	+	-	GC	-	-	++	-
10	Diff centro	-	-	+	Non-GC	++	+	-	++
11	Centro-multi	+	++		GC	-	+	-	+
12	Centro-multi	-	+	-	GC	-	+	-	+
13	Diff centro	+	-	-	GC	-	-	++	++
14	Diff centro	-	+		Indet	-	+	-	+
15	Centro-multi	-	+		Indet	-	+	-	-
16	Diff centro	-	+		Indet	-	+	-	+
17	Centro-multi	-	-		Non-GC	-	+	-	++
18	Diff centro	+	++	+	GC	-	+	-	++
19	Centroblastic	-	+	-	GC	-		-	-
20	Immunobl	-	++	+	Non-GC	++	-	-	-
21	Centro-multi	++	++	-	GC	-	+	-	++
22	Centro-multi	-	-	-	Non-GC	-	-	-	++
23	Centro-multi	++	+		GC	++	+	-	++
24	Centro-multi	+	-		GC	++	-	-	++
25	Centro-multi	-	+	-	GC	-	-	++	++
26	Centro-multi	-			Indet	++	-	-	++
27	Centro-multi	-	++	-	GC	-	+	-	-
28	Diff centro	++	+	+	GC	++	-	++	++
29	Centroblastic	-	-	+	Non-GC	++	-	-	++
30	Diff centro	-	++		Indet	++	-	++	++
31	Diff centro	++	+	+	GC	-	++	-	++
32	Centro-multi	-	++	-	Indet	-	+	++	+
33	Centro-multi	-	+	-	GC	-	-	-	++
34	Centro-multi	-	+	+	Non-GC	-	++	-	++
35	Centro-multi	++	+	-	GC	++	-	-	+
36	Centro-multi	+	++	-	GC	-	-	-	++

-: no staining

+: 30 to 75% of the tumour cells positive staining

++: more than 75% of the tumour cells positive staining

(n=26) was 90%, versus 40% for those patients who were over 60 years of age at the time of diagnosis (n=10; p-value=0.0004) (figure 1). The patients who presented with multiple bone lesions (n=10) at the time of diagnosis had a significantly worse survival than those who presented with a lymphoma at a single location (n=26), the overall 5-year survival being 41% versus 90%, respectively (p-value=0.0085) (figure 2).

### **Histological and Immunohistochemical Features (table 3)**

The histological and immunohistochemical findings are summarized in table 3. Although not recognized anymore as separate tumour entities in the present WHO, a morphological subclassification was performed in order to establish whether a specific morphology was apparent. Twenty cases demonstrated centroblastic-multilobated morphology, 13 cases showed centroblastic mono- or polymorphic morphology, two cases showed centroblastic-centrocytic morphology and one case demonstrated immunoblastic morphology. No prognostic significant difference in survival between the morphological tumour subtypes was found, nor did we find a difference in morphological subtype between the stage I and IV patients.

Nuclear expression of BCL-6 was seen in 28 cases. BCL-2 expression was demonstrated in 12 cases, 8 cases showed both BCL-2 and BCL-6 expression. CD10 expression was seen in 13 cases. MUM-1 expression was found in 8 out of 22 cases which could be evaluated. P53 expression was found in 16 cases. CD30 expression was seen in 7 cases, CD44 expression was seen in 29 cases.

Applying the Hans' algorithm, GC phenotype was defined as CD10+BCL-6+, non-GC phenotype was defined as CD10-BCL-6-. In case of CD10-BCL-6+, MUM-1 expression was determined. [14] If MUM-1 was negative, the phenotype was defined as GC, if MUM-1 expression was positive, the case was defined as non-GC. If MUM-1 expression was unavailable, the phenotype was defined as indeterminate. Nineteen out of 36 patients revealed a GC phenotype. Tumours that were negative for both CD10 and BCL-6, the non-GC phenotype, were seen in 8 cases. The phenotype was defined as indeterminate in 9 cases.

There was no significant difference in survival between the different tumour phenotypes. None of the other antigens tested had a significant association with survival.

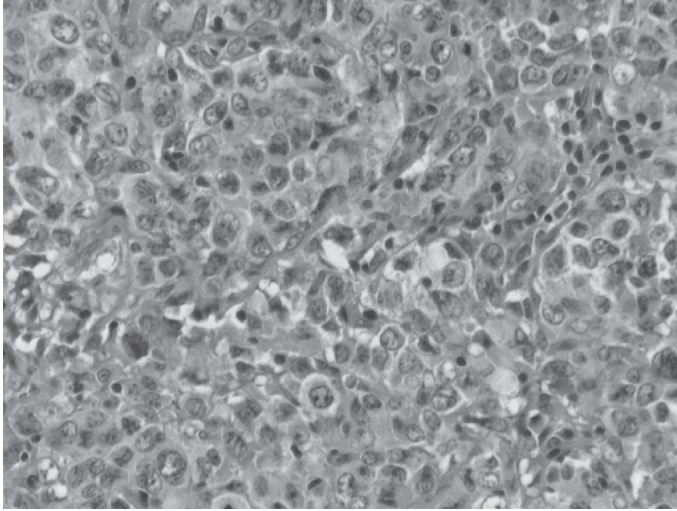
## **Discussion**

Our study of PLB investigated immunohistochemical expression of BCL-6, CD10, BCL-2, MUM-1, p53, CD30 and CD44 to analyse the prognostic significance of these markers and to determine GC derivation.

Patients displayed homogeneous clinical features, consistent with the few other reports on PLB in literature, but not earlier was the homogeneity as strong as in the present cohort. [2,12,15-19]

Similar to other series the presenting symptom in all patients was pain, usually combined with a palpable mass; the site of presentation was mostly in the long bones and the age at presentation was in the fifth decade. The histology was consistently compatible with DLBCL and displayed predominantly a centroblastic morphology with multilobated nuclei.

The PLB patients had a good prognosis, with a complete remission rate of 80% and an overall survival of 75%. Only one patient suffered a relapse, which was followed by complete remission. Our series included 10 cases of stage IV disease with multiple bone localizations, with a 5-year overall survival of 41%, whereas most prior studies excluded patients with multiple



**Figure 3.** A typical case of primary lymphoma of bone showing centroblastic cells with nuclear multilobated features.

bone involvement, which leads to an imperfect representation of the spectrum of this disease.

Two IPI risk factors, age at presentation and stage of disease, were of significant influence on 5-year overall survival, with a survival rate of 90% for the patients under 60, and 40% for those of 60 years and older. Survival rates were 90% for stage I vs 41% for stage IV, respectively.

In recent studies, the relationship of B-cell malignancies to normal stages of B-cell differentiation and activation has been partly clarified using genomic scale gene expression profiling. These molecular distinctions between subgroups of DLBCL are important because a difference in response to multi-agent chemotherapy is noted between these subgroups, with a favourable outcome for the GC phenotype. The Hans' algorithm is the most widely accepted algorithm to define phenotype using immunohistochemistry. Previous studies on PLB confirmed the positive effect on survival for the GC phenotype, whereas a worse survival in case of lack of CD10 expression, presence of MUM-1 expression and non-GC phenotype was found.[11,12]

The p53 tumour suppressor gene product is expressed in 20 to 50% of DLBCL. Protein expression in DLBCL is associated with treatment failure and poor outcome. In this study p53 expression was found in 16 of 36 cases, without a significant effect on survival.[20] The intrinsic capacity of CD44+ tumour cells to disseminate does not appear to play a role in PLB, as all positive cases were localized to the bone without evidence of extra-osseous involvement and without an adverse effect on prognosis.

In our study we could not demonstrate a statistically significant influence on prognosis of tumour phenotype, nor did we find a significant effect on prognosis by the individual immunohistochemical markers. We think this difference between our results and those of others is explained partly by the favourable survival of our cohort, with only five tumour related deaths and only one relapse out of 36 patients. Another difference between our cohort and that of others is the striking homogeneity, with very similar morphology, phenotype and clinical

course for the majority of the cases. This positive and homogeneous clinical course results in few statistical events. Remarkably however, 50% of the patients with progressive disease had a non-GC phenotype. Furthermore, the one patient with a relapse had an immunoblastic morphology and was of the non-GC phenotype. In a previous study, a similar negative correlation with survival was noted for the immunoblastic phenotype.[2]

Of note, all recent PLB studies found a clear morphologic and phenotypic relation of PLB to GC cells, despite the fact that normal bone or bone marrow lacks germinal centres. These findings support the hypothesis that PLB represents a *de novo* DLBCL with a genetic relationship to GC like lymphomas. Some authors speculate that there are specific homing factors for these tumour cells. More research is necessary to investigate the pathophysiology of this rare extranodal subtype of DLBCL.

In conclusion, this study illustrates the homogeneity of PLB. The majority of cases is of the GC phenotype and has a favourable prognosis.

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