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A Clinicopathologic Study on the Diffuse Malignant Lymphoma

—A morphologic and immunophenotypic analysis in 62 patients at Harbor-UCLA Medical Center—

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In order to compare the prognoses of patients with diffuse malignant lymphomas on the basis of histology and immunophenotypes, we retrospectively studied 62 cases of diffuse lymphoma arising in lymph nodes. We also evaluated the reactivity patterns of monoclonal antibodies (MoAb) LN1, LN2 and LN3 to determine the criteria for making a differential diagnosis in B cell lymphomas. The immunologic phenotypes were determined by the avidin biotin peroxidase complex method, using frozen or paraffin fixed tissues. The majority (66.3%) were B cell with the remaining 20.9% being T cell and 12.9% were non-B, non-T cell lineage. Immunological heterogeneity was found especially in the mixed small and large cell and the immunoblastic lymphomas. There was no significant difference between B-and T-cell lymphomas with respect to survival and death ($P>0.05$). Histologically 79% (49/62) of the lymphoma was large cell and 21% (13/62), small cell lymphoma. There was a difference in prognosis between low, intermediate and high-grade of lymphomas. However there were no significant differences among the subtypes of the diffuse aggressive lymphomas. Factors associated with poor prognosis were advanced stages ($P<0.025$) and histology of the malignant lymphomas. MoAb LN1, LN2 and LN3 gave positive staining in 83.3%, 91.7% and 60% of B cell lymphomas, respectively. The most common phenotypic pattern in B cell lymphomas was LN1+, LN2+, LN3+/−, suggestive of follicular center cell origin. As a panel, phenotypic patterns of MoAb LN1, LN2 and LN3 may be useful in differentiation of follicular center cell lymphoma from others.

Key Words: *Diffuse lymphoma, Immunophenotype, Morphology, Prognosis*

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a diverse group of malignant lymphoproliferative disease that differs with regard to histologic appearance, mode of presentation, clinical behavior and response to therapy (Sun 1988). Over the past few decades, substantial efforts have been made to determine the characteristics in individual tumors which predict clinical behavior and prognosis. To date, the main prognostic factor has been histologic classification (NCI, 1982 Simon et al., 1988). Despite ongoing efforts to refine the histologic criteria, the diagnosis and classification of malignant lymphomas is still a challenge to many pathologists. This is probably due to the limitations and ambiguities of morphology and immunophenotypes. Even though

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there is a controversy as to whether immunophenotypes and histologic subtypes of the malignant lymphoma are clinically relevant, there is, however, a continuing need to further analyze the spectrum of NHLs.

Recently, the development of monoclonal antibodies (MoAbs) reactive with lymphoid and monocytic subsets (Chan et al., 1988 Picker et al., 1987), has permitted the improvement of classification, primary diagnosis, and clinical staging of lymphoid neoplasm (Stein et al., 1980). Since processing of tissue for paraffin embedding often significantly alters antigenic determinants, most antibodies are used on viable cell suspensions or frozen sections. However, several very useful antibodies do react with formalin or B5 fixed, paraffin embedded tissue (Ng et al., 1987, Norton and Isaacson 1987, 1989, 1989, Okon et al., 1985). The immunologic analysis on fixed tissue in paraffin sections provides superior morphology and allows for the conservation of sometimes limited biopsy materials (Andrade et al, 1988, Kurtin et al., 1985 Norton and Isaacson 1989, 1989).

Using multiple immunologic and enzyme histochemical assays, authors have determined the immunologic phenotypes of diffuse lymphomas arising in lymph nodes. We have subgrouped the diffuse lymphomas into two categories; diffuse lymphoma of small lymphoid cells and diffuse aggressive lymphomas (Cossman et al., 1984).

The purpose of this study is to compare the prognosis of the diffuse NHLs on the basis of mophology and immunophenotypes. In addition, immunoperoxidase studies were performed using Mo Ab LN1 (CDw75), LN2 (CD74) and LN3 which identify follicular center cells and B cells in B5 fixed tissue sections. The immunostaining pattern of these antibodies was compared for the purpose of establishing the criteria for making a differential diagnosis between histologic subtypes of B cell lymphomas.

MATERIALS AND METHODS

Case Selection

Sixty two cases of diffuse malignant lymphomas that had been studied by immunohistochemistry and enzyme histochemistry, were retrieved from the files of the Department of Surgical Pathology at the Harbor-UCLA Medical Center. All cases were diagnosed during the years from 1982 to 1990. We selected cases on the basis of the availability of adequate paraffin tissue and clinical informations. Histologic diagnoses were made on the basis of hematoxylin and eosin stained paraffin sections and were reviewed by two of the authors. Histologic classification was done according to the criteria of a modified Working Formulation (Burke 1990, NCI 1982). Four patients included in this study had a prior diagnosis of follicular small cleaved or follicular mixed small and large cell lymphoma from which a histologic transformation had occured. Also included is one case of small lymphocytic lymphoma which was converted to large cell lymphoma, known as Richter syndrome (Haroussear et al., 1981).

Immunologic phenotyping studies

Immunologic phenotypes were determined by the avidin biotin peroxidase complex method (Hsu et al., 1981) using touch imprints, frozen section, and the B5-or formalin-fixed materials.

The list of antibodies used on the paraffin tissues, was LN1 (CDw75), LN2 (CD74), LN3, UCHL-1 (CD45RO), L26, Leu22 (CD43), MT1 (CD43), MB2, Kappa, Lambda, Leu M1 (CD15), Ber-H2 (CD30), lysozyme, keratin, S-100, and EMA.

The list of antibodies used on frozen tissue section and/or touch imprints, was Ia (I2), B1 (CD20), B4 (CD19), J5 (CD10), T11 (CD2), T4 (CD4), T8 (CD8), T3 (CD3), Kappa, Lambda and T200 (CD45).

Under the standardized method in the laboratory (Sun 1983), enzyme cytochemistry was done on touch imprints: acid phophatase (with and without tartrate inhibition), alpha-naphthyl acetate esterase, alpha-naphthyl butyrate esterase, beta-glucuronidase and alkaline phosphatase.

Immunofluorescence for nuclear terminal deoxynucleotidyl transferase (Tdt) was also done.

Clinical Parameters

Sixty-two patients have been analyzed in terms of the clinical characteristics or survival. The patients were staged in accordance with the Ann Arbor system (Moormeier et al, 1990). Four patients with small lymphocytic lymphoma associated with chronic lymphocytic leukemia were staged in accordance with the Rai system (Rai et al., 1975).

Combination chemotherapy regimens utilized in these patients included: M-BACOD (Methotrexate, Bleomycin, Deoxyrubicin, Cyclophophamide, Vincristine, Dexamethasone), CHOP (Cyclophosphamide Adriamycin, Vincristin, Prednisone), VP-16, ESHAP, CVP (Cyclophosphamide, Vincristine, Prednisone) and COM-LA (Cyclophosphamide, Vincristine, MTX, Leucovorin, Cytarabine). CHOP chemotherapy was used in 14 patients, 2 of whom also received the M-BACOD. Eleven

patients received M-BACOD regimens. Sixteen patients received radiation therapy combined with chemotherapy. Survival was calculated from the start of therapy until death.

Statistical analysis was done by a Chi-Square test.

RESULTS

Immunologic Analysis

The histologic subtypes and results of immunologic phenotypes in the 62 cases of diffuse lymphomas were shown in Table 1. The cell of origin could be defined in 54/62 (87%) of diffuse lymphomas. Of the 54 cases, 41 (66.1%) cases had B-cell markers and 13 (20.9%) had T-cell markers. Eight cases could not be determined and classified as non-B and non-T, based on the lack of demonstrable surface markers and/or mixed ambiguity in immunophenotypes.

Correlation of immunophenotypes with histologic subtypes in the 62 cases of diffuse lymphoma revealed that the malignant lymphomas of large cell (17/20),

Table 1. Immunophenotype of Diffuse Malignant Lymphomas

Histologic Subtypes	Number of Case	B	T	non-B non-T
Large	20	17		3
Mixed	6	3	3	
Immunoblastic	4	2	2	
SNC	8	8		
Lymphoblastic	9		7	2
Ki-1	2			2
SL (w/CLL)	5	3	1	1
ILL	5	5		
SCC	3	3		
Total (%)	62	41(66.1)	13(20.9)	8(12.9)

SNC: small noncleaved lymphoma, SL (w/CLL): small lymphocytic lymphoma (with chronic lymphocytic leukemia), ILL: lymphocytic lymphoma of intermediate differentiation, SCC: malignant lymphoma, small cleaved cell

Table 2. Clinical Summary of Diffuse Malignant Lymphoma

Histologic Subtypes	Number of Case	Age Median	Sex M:F	Advanced Stage (%)	Death Case (%)	Survival Median (M)
Large	20	43.5	13:7	14/20(70)	11/20(55)	15.0
Mixed	4	46.5	1:1	4/4(100)	4/4(100)	19.5
Immunoblastic	4	24.5	1:1	3/4(75)	2/3(66.7)	14.0
SNC	8	43.0	5:3	7/8(87.5)	4/7(57.1)	2.0
Lymphoblastic	9	24.0	6:3	5/9(55.6)	5/9(55.6)	17.0
Ki-1	2	17.5	2:0	2/2(100)	—	—
AILD-like	1	62.0	1:0	1/1	—	—
PTC	1	65.0	1:0	1/1	—	—
SL (w/CLL)	5	48.0	3:2	1/5*	—	—
ILL	5	46.0	3:2	5/5(100)	—	—
SCC	3	74.0	1:2	3/3(100)	2/3(66.7)	168
Total (%)	62	43.5	1.8:1	46/62(74.2)	28/59(47.5)	

*Rai system

Mixed: malignant lymphoma, mixed small and large cell, SNC: small noncleaved lymphoma, AILD-like: angioimmunoblastic lymphadenopathy like lymphoma, PTC: peripheral T cell lymphoma, SL (w/CLL): small lymphocytic lymphoma (with chronic lymphocytic leukemia), ILL: lymphocytic leukemia of intermediate differentiation, SCC: malignant lymphoma, small cleaved cell
M: months

small non-cleaved cells (8/8), small lymphocyte (3/5), intermediate lymphocyte (5/5) and small cleaved cells (3/3) were almost exclusively of B cell type (36/14, 87.8%).

Mixed small and large cell type (with or without epithelioid cells) and immunoblastic types were immunologically heterogenous. Half of the cases (5/10) were of B cells and another half were of T cells of peripheral or post-thymic T lineage. Each case of angioimmunoblastic lymphadenopathy like lymphoma and peripheral T cell lymphoma was included in the mixed small and large cell lymphoma by the Working formulation. They were all of T cell lineage.

Lymphoblastic lymphomas were exclusively T-cell type of thymic origin except in 2 cases.

Non-B, non-T cell lymphomas contained 3 cases of large cell lymphoma, 2 cases of Ki-1 lymphoma, 2 cases of lymphoblastic lymphomas and one case of small lymphocytic lymphoma. One case of lymphoblastic lymphoma classified as non-T, non-B cell lineage may be a pre-B based on the positive for B4 (CD19). Also 25% of lymphoid cells were positive for T11 (CD2) and T4 (CD4).

Clinical correlation and Follow-up

Table 2 showed the clinical characteristics of 62 patients according to histologic subtypes.

The median age of 62 diffuse malignant lymphomas was 43.5 (range, 11-87). Ki-1 lymphomas and lymphoblastic lymphomas characteristically occured in an adolescent and in a young adult (median age, 17.5 and 24.0). Also immunoblastic lymphoma was presented in the younger age of 24.5. This is probably due to including one case of ataxia-telangiectasia associated with malignant lymphoma.

The sex ratio (M:F) was 1.8:1 with a slight male predominance in diffuse malignant lymphomas.

The majority of the patients (46/62, 74.5%) had stage III and IV disease at the time of diagnosis. Advanced stage was reported especially in mixed small and large cell, small noncleaved, Ki-1, small lymphocytic, intermediate lymphocytic lymphoma and small cleaved cell lymphoma. Four cases of small lymphocytic lymphoma with chronic lymphocytic leukemia all involved bone marrow and lymph nodes and were classified as stage I according to the Rai system (1975).

Fourty seven percent of the patients (28/59) died of diffuse lymphomas. Three cases lost follow up and were excluded from the analysis. The median survival from the initiation of therapy was comparable with the histologic subtypes: 15 months (M) for large cell lymphoma, 19.5M for mixed, 14M for immunoblastic, 2M for small noncleaved, 17M for lymphoblastic lymphoma and 168M for small cleaved cell type.

There was a significant difference in the survival time between the two groups of diffuse large, aggressive and small cell lymphomas. We could not find any significant differences in the survival among the histologic subtypes of diffuse aggressive lymphoma. The one exception was patients with small noncleaved cell lymphoma who had the shortest median survival of 2 months. This was probably due to 3 cases of AIDS.

Clinicopathologic correlation between staging, histologic subtypes and immunologic types

Clinicopathologic correlation of staging is revealed

Table 3-1. Statistical Analysis of Staging and Death Cases

Stage	I-II	III-IV	Total
Death	3	25	28
Alive	12	29	31
Total (%)	15(20)	44(56.8)	59

( ): Percentage of Death Case ($P<0.025$)

Table 3-2. Statistical Analysis of Histology and Death Cases

Grade	Low	Intermediate	High	Total
Death	0	17	11	28
Alive	9	12	8	29*
Total (%)	9(0)	29(58.6)	19(57.9)	57

*Exclude Ki-1 lymphoma

in Table 3-1. 25 of 44 cases with advanced stages (stage III and IV), died of diffuse malignant lymphomas, compared with only 20% of the early stage (Chisq=5.714, df=1 $P<0.025$). There was a statistically significant difference between these two groups of advanced and early stage. However patients with Ki-1 lymphoma, small lymphocytic (with or without CLL) and intermediate lymphocytic lymphoma had advanced stage and were all alive.

We compared the death cases according to the low, intermediate and high grade lymphomas (Table 3-2). There was a significant difference in the death cases between each group. However we did not find any significant difference for the following comparison; intermediate versus high grade lymphoma (Chisq=0.12, df=1, $P>0.05$).

According to the immunologic phenotypes of diffuse malignant lymphoma (Table 4), 44% of B-cell lymphoma (17/39), 61.5% of T-cell lymphoma (8/13) and 42.9% of so called non-B, non-T lymphoma (3/7) died of the disease. There were no significant differences among them (Chisq=0.894, df=1, $P>0.05$). Median survival was comparable for the immunologic phenotypes; 1 year for 17 cases of B cell lymphomas, 2 year for 8 cases of T cell lymphoma and 1 year for 3 cases of non-B, non-T lymphomas. There were also no significant differences in the survival among them.

Expression of LN-1 (CDw75), LN-2 (CD74) and LN-3 in B cell lymphomas

The light chain restriction was present in 88.2% (30/34) of B cell lymphoma. Kappa monoclonality was present in 17 (50%) of 34 cases and lambda monoclonality, in 13 cases (38.2%). An excess of cases expressing lambda light chain was noted (kappa-lambda ratio of 1.3; normal 2.0).

The expression of LN-1, LN-2 and LN-3 in B cell lymphoma is shown in Table 5. LN-1 was positive in 83.3% (30/36) of B cell lymphomas. LN-1 reacted most strongly with the large cell, mixed small noncleaved and small cleaved cell lymphomas derived from follicular center cell. It was weakly positive or negative in cells of the small lymphocytic and intermediate lymphocytic lymphoma. One case of small lymphocytic lymphoma revealed a focal staining of LN-1 in the proliferation centers

Table 4. Correlation of Immunologic Type and Death

	B	T	non-B, non-T
Death	17	8	3
Alive	23	5	4
Total (%)	39(43.6)	13(61.5)	7(42.9)

( ): Percentage of Death Case ($p>0.05$)

Table 5. Immunoreactivity of LN1, LN2 & LN3 in Diffuse B Cell Lymphomas

Histologic Subtype	Case	LN1	LN2	LN3	Kappa	Lambda
		(Positive case/Total case)				
Large	17	17/17	15/17	7/13	11/17	5/17
Mixed	3	3/3	3/3	1/3	1/3	1/3
Immunoblast	1	1/1	1/1	1/1	1/1	—
SNC	7	7/7	7/7	3/5	3/7	4/7
SL (w/CLL)	2	1/2	1/2	2/2	—	1/1
ILL	5	0/5	5/5	3/5	1/5	2/5
SCC	1	1/1	1/1	1/1	ND	ND
Total (%)	36	30/36(83.3)	33/36(91.7)	18/30(60.0)	17/34(50)	13/34(38.2)

ND: not done

Mixed: malignant lymphoma mixed small and large cell, SNC: small noncleaved lymphoma, SL (w/CLL): small lymphocytic lymphoma (with chronic lymphocytic leukemia), ILL: lymphocytic lymphoma of intermediate differentiation, SCC: malginant lymphoma, small cleaved cell

Table 6. Patterns of LN1, LN2 & LN3 in Diffuse B Cell Lymphomas

Patterns			Histologic	Subtype	(Positive Case/Total Case)		
LN1/2/3	Large	Mixed	Immunoblast.	SNC	SL (w/CLL)	ILL	SCC
+/+/+	7/17	1/3	1/1	3/7	—	—	1/1
+/+/–	8/17	2/3	—	4/7	—	—	—
+/–/–	2/17	—	—	—	—	—	—
–/+/+	—	—	—	—	1/2	3/5	—
–/+/–	—	—	—	—	—	2/5	—
+/–/+	—	—	—	—	1/2	—	—

Mixed: Malignant lymphoma mixed small and large cell SNC: Small non cleaved lymphoma
SL (W/CLL): Small lymphocytic lymphoma (with chronic lymphocytic leukemia)
ILL: Lymphocytic lymphoma of intermediate differentiation SCC: Malignant lymphoma, small cleaved cell

except small lymphocytes. LN-1 staining was localized to the cell membrane and cytoplasm. Of 30 cases, 12 cases revealed an unusual paranuclear cytoplasmic dot-like staining of LN-1, suggesting the presence of an abundance of the antigen in Golgi region of the neoplastic cells. This paranuclear staining was seen in the large cell lymphoma and one case of mixed cell types. The LN-2 reacted strongly in 91.7% (33/36) or B-cell lymphomas. The LN-2 staining was localized to nuclear membrane and often cytoplasm. A paranuclear cytoplasmic dot like staining of LN-2 was present in 5 of 33 cases. The LN-3 was positive in 60% (18/30) of B cell lymphoma. The LN-3 bound to the surface membrane and the cytoplasm.

The phenotypic patterns expressed by LN1, LN2 and LN3 were summarized in Table 6. The most common pattern of B cell lymphoma was LN1+, LN2+ LN3+ and LN1+, LN2+, LN3– in 36.1% and 38.9% respectively. The intermediate lymphocytic lymphoma and small lymphocytic lymphoma with or without CLL revealed chracteristically LN1–, LN2+, LN3+/– pattern.

DISCUSSION

The clinical, morphological and immunological heterogenity of the diffuse lymphoma has been emphasized in previous publications (Doggett et al., 1984, Freedman et al., 1985, Lennert et al., 1975, Lukes and Collins 1974, Stein et al., 1980, Strauchen et al., 1978). Intratumoral heterogeneity may reflect growth or differentiation differences between subpopulations of individual neoplastic clones. The distinction between histologic subtypes of diffuse lymphoma is important to the clinician, since the subclasses convey different biologic behavior. In our study, the diffuse lymphomas divided into two categories based on the Rosenberg's study (1979); favorable or small cells, and unfavorable or large cells. The unfavorable lymphomas have an aggressive natural course and at the same time sensitivity to chemotherapeutic agents rendering the possibility of a cure in a significance number of patients (Cossman et al., 1984). Our study found that the majority (49/62, 79%) of the diffuse lymphoma was large cells and aggressive lymphoma and 13 cases (21%) were of small cell lymphoma.

We examined the immunophenotypes in 62 lymphomas. The majority were of B cell (66.3%) with the remaining 20.9% being of T cells and 12.9% of non-T non-B lineage. In the review of 425 cases of malignant lymphoma (Lukes et al., 1978) the functional studies reported 68.2% of B cell, 18.7% of T cells and 12.9% of undefined cells. The distibution of immunophenotypes was very similar to ours.

In our study, the malignant lymphomas composed of small lymphoid cells were predominantly of B cells except for two cases of small lymphocytic lymphoma. Lymphoblastic lymphoma was predominantly of T cell lineage except in two cases. One of them might be of pre-B cell origin based on diffuse staining for B4. Although lymphoblastic lymphomas and small lymphocytic lymphomas are morphologically homogenous, they were also reported to be immunologically heterogenous like our results (Cossman et al., 1983, Tosi et al., 1990).

The diffuse mixed small and large cell lymphomas were well known as an immunologically and morphologically heterogenous type (Katzin et al., 1989). It included diffuse counterpart of follicular mixed small and large cell lymphoma as well as difuse mixed lymphoma of T cell origin, such as angioimmunoblastic lymphadenopathy like lymphoma and Lennert's lymphoma. One case of peripheral T cell lymphoma

in our study may be classified as a mixed small and large cell lymphoma by the Working Formulation.

The large cell lymphomas were presumably of follicular center cell origin and were predominantly of B cell origin. Large cell, immunoblastic lymphomas were also heterogenous and included both B-cell and T-cell lymphomas. However, Burkitt's lymphoma and Burkitt's type lymphoma were always of B cell.

Cossman et al (1984) reported that the immunophenotype of diffuse aggressive non Hodgkin's lymphoma could be determined in 97% among the examined cases; 53% was B-cell type, 42% were peripheral T cell type and one was true histiocytic. Doggett et al (1984) reported 89 patients with nodal and extranodal diffuse large cell lymphoma using cryostat sections. They found 53% was B cell, 14% was T cell and 31% was non-T, non-B cell. Freedman et al (1985) also reported immunologic heterogeneity of diffuse large cell lymphoma with 82% of B cell, 16% of T cell and 2% of monocyte-myeloid lineage.

Next, we questioned whether there is any prognostic significance among the immunophenotypes? An early report of surface marker analysis stated that immunologic phenotype was an important prognostic factor in non-Hodgkin's lymphoma.

Bloomfield et al., (1979) have reported that patients with B-cell lymphomas are more likely to achieve long-term survival than those with T-cell lymphomas and that patients whose lymphomas are null have the worst prognosis. In that study, the majority of B-cell lymphomas were low grade. All but one of the T-cell cases in that study were lymphoblastic lymphomas and most of the null cell lymphomas were from high grade histologic categories.

A more morphologically homogenous group was studied by both Warnke et al., (1980) and Rudders et al., (1981). Warnke's group (1980) reported a series of large cell lymphoma of non T type and identified to phenotypically different types; HLA-DR+, sIg+ and HLA-DR+, sIg−, T−. They stressed the importance of distinguishing groups, since the sIg− group had a better prognosis and responded better to therapy than did the other group.

Rudders and others (1981) were unable to demonstrate definitive surface markers in 57% of cases and only 1 of 31 was of T-cell type. Although actuarial survival was significantly better for the B-cell group, the overall complete remission rate was only 32% (10/31) and was similar for the null (32%) and B-cell (30%) groups.

The immunologic phenotype did not appear to significantly influence the survival of patients with diffuse, aggressive, non-Hodgkin's lymphomas (Cossman et al., 1984) and immunoblastic sarcomas (Levine et al., 1981).

Horning and others (1984) concluded retrospective cell phenotying analysis had not provided independent prognostic significance in 78 diffuse large cell lymphomas. Forty one lymphomas (53%) expressed immunoglobulin. Of the 37 cases that did not express immunoglobulin, 9 expressed T cell antigen. The actuarial survival among patients with Ig− lymphomas was superior to that of Ig+ patients. However, this difference was explained by a higher proportion of Ig+ patients with unfavorable prognostic facotrs, such as advanced stage disease, age more than 65 years, and systemic symptoms.

Comparison of all of these reports is difficult, because they involved differences in technique, small patient numbers, different therapies and they failed to take into account prognostic variable that are known to affect survival.

While the prognostic significance of T-cell or B-cell origin continues to be debated in the literature, our study also appeared that the cell of origin did not influence the survival and the prognosis (Table 4, $p > 0.05$).

Schuurman and others (1988) reported CD23 expression was associated with a longer disease free survival in a low grade malignancy. They also concluded the detailed immunologic phenotyping has relatively little prognostic value when compared with that of the malignacy grade by conventional histopathology.

Factors associated with poor survival are advanced stage, bone marrow involvement, increased bulk of disease, advanced age, systemic symptoms and others (Cabanilla et al., 1978, Fisher et al., 1981, Fisher et al., 1977, Taylor 1979). The histologic classification has long been of greatest prognostic value (NCI, 1982, Simon et al., 1988). Long term prognosis depend on the histologic subtype of the tumor and the extent of dissemination. Our study revealed that advanced stage was significantly related to the poor prognosis (Table 3-1, $p > 0.025$). There was no patient with low grade lymphoma who was dead in our study, although the period of follow up was short (from 1 year 8 months to 4 years). Although we did not statistically analyze it, there was a significant difference in survival time and number of death cases between low-, intermediate and high grade of NHL (Table 3-1).

But, we found there was no significant difference between intermediate and high grade of the lymphoma ($p > 0.05$). The Southwest oncology group (Nathwani et al., 1982) studied the clinical significance of the morphologic subdivision in 162 patients with diffuse histio-

cytic lymphoma and reported that there was not any siginificant difference in survival among them. We also questioned whether or not the histologic subtypes of the diffuse aggressive lymphoma were clinically and prognostically relevant, in spite of the fact the patients with small noncleaved cell lymphoma had the shortest survival. However many investigators have shown that clinical differences among these subgroups have been documented (Strauchen et al., 1978, Fisher et al., 1981).

Our patients with certain histologic types of the malignant lymphoma such as Ki-1, AILD like lymphoma and peripheral T cell lymphoma had advanced stage and were still alive. Although there were too few cases to come to any conclusion, Ki-1 lymphoma in the childhood cases was reported to have a high cure rate with aggressive therapy (Schnitzer et al., 1988).

In 1984, Epstein and others reported the use of two antibodies (LN1, LN2) reactive with B cell in paraffin sections. Subsequent studies have confirmed these findings and LN1 & LN2 have been to stain 88% and 92-95% of B cell lymphoma respectively using B5-fixed tissues (Marder et al., 1985, Okon et al., 1985).

Our study showed that these antibodies gave postive results in 83.3% and 91.7% of the B cell lymphoma respectively.

However, Ng et al, (1987) reported that LN1 & LN2 were positive in 44.9% and 46.4% of formalin fixed B cell lymphoma, respectively. They interpreted that this results appeared to be inferior to that obtained in B5 fixed tissue.

LN1 is strongly positive in germinal center B lymphocytes and is negative or very weakly reactive with mantle zone B lymphocytes. T cells and histiocytes are negative. LN-1 is also positive on a variety of epithelial cells including cells of the renal tubules, breast, bronchus and prostate (Epstein et al., 1984, Marder et al., 1985, Okon et al., 1985). In our study, LN1 was positive in the diffuse small cleaved, large cell, mixed small and large, and small noncleaved types. These findings support that these neoplasms are derived from the follicular center cells. Although there is a controversy (Norton and Isaacson 1989, Shepherd et al., 1988, Hall et al., 1988), LN1 is still useful as it highlights the follicular center cells and derived tumors (Ng et al., 1987, Norton and Isaacson 1987, Samoszuk et al., 1986).

One case of immunoblastic sarcoma stained with LN1 in our study needed to be explained. The immunoblasts, a term initially proposed by Dameshek (1973) are thought to be derived from the extrafollicular transformed cells for the precursor of cellular immune proliferation (Florentin, 1975). Conceptually, LN1 should be negative in immunoblastic lymphoma. But Norton and Isaacson (1987) also reported 8 of 10 immunoblastic lymphoma were positive with LN1, identical to our result. This may suggest that immunoblastic lymphoma may have some relationship to the follicular center cells.

Another explanation is that there may be histologic overlap between large cell and immunoblastic lymphoma (Stein et al., 1984). Despite the fact that the criteria for dividing large cell and immunoblastic lymphoma are ostensibly precise, such discriminations are frequently difficult.

Interesting was one case of small lymphocytic lymphoma with CLL which revealed focal positive staining of LN1: the proliferation centers were stained, whereas none of small lymphocytes were reactive. Our result is identical to others in which LN1 was stained in 5 out of 8 cases of small lymphocytic lymphoma with CLL (Ng et al., 1987).

LN2 is positive in the nuclear membranes of B cells in the mantle zone and germinal centers. Histiocytes, Reed-Sternberg cells and various non-hematopoietic cells also have been reported to express LN2 antigen (Epstein et al., 1984, Marder et al., 1985, Okon et al., 1985).

LN3 (Marder et al., 1985) is a polymorphic HLA-DR (Ia like) antigen. The HLA-DR antigen is present on germinal center and mantle zone B cells, monocytes, macrophages and interdigitating histiocytes. T cells are generally negative although activated T cells may express HLA-DR. Our studies showed LN1 and LN2 appeared to be more consistently expressed than LN3 in B cell lymphomas.

Because Ia may be expressed on activated T cells and T cell neoplasm (Aisenberg et al., 1983, Halper et al., 1980, Reinherz et al., 1979), LN2 or LN3 positivity alone must be viewed cautiously in making an absolute diagnosis of B cell lymphoma. Ng et al (1987) reported that LN1 and LN2 gave false positive results in a few cases (3.6% and 5.5%) of T cell lymphoma.

However when Mo Ab LN1 and LN2 were used as a panel, the immunophenotypic patterns would be useful in histologic classification of B cell lymphomas. As in our study, most B cell lymphoma revealed a pattern of LN1+, LN2+ and LN3+/− suggesting follicular center cell origin. The intermediate lymphocytic lymphoma and small lymphocytic lymphoma with or without CLL showed LN1−, LN2+ and LN3+/−.

In summary our results suggest that immunological characterization of lymphomas provides additional information which may supplement the morphological classification. It should be emphasized that there is no

good substitute for a morphological histologic classification, regarding the prognostic implication.

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