CHAPTER 2

REVIEW OF RELATED LITERATURE

2.1 Historical Overview

The term "malignant lymphoma" was originally introduced by Billroth to describe neoplasms of lymphoid tissue[23]. It has been traditional to divide lymphomas into Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) because of their difference in histology and pattern of behavior. Whereas HD is a single disease entity, the NHL represents a more separate, though related, disorders with distinct prognoses, clinical pictures and treatment requirements. Identification of these separate entities depends largely on the discipline of hematopathology and forms a major criterion of prognoses and management.

In his textbook published in 1863, Virchow was the first who introduced the term "lymphosarcoma" describing patients with "aleukemic" form of leukemia[24]. It was well into the twentieth century that the term follicular or nodular lymphomas were first clearly described by Brill et al in 1925[25]. However, it was only with the classic study of Rappaport et al in 1956 that medicine was presented with a lymphoma classification that could be easily applied and was prognostically useful[26]. The important concept of follicle center cell as the major fraction of adult NHL was reinforced by Lennert, Lukes and Collins[27, 28]. The most important classification for clinical usage however was the Working Formulation introduced by the National Cancer Institute in 1982[3]. The system had gained wide popularity because it divided patients into three prognostic groups according to the histopathology, low-, intermediate- and high-grade. However as the availability of immunohistochemistry has merged into medicine in the late 1980s, the

nature of many lymphoid proliferations were illuminated and yet another more relevant classification for NHL has to be developed.

The evolution of effective treatment for NHL stemmed from the invention of curative chemotherapy regimen, MOPP (nitrogen mustard, oncovin, procarbazine, prednisolone), for patients with HD in 1970[29]. Five years later, the first era of cure in patients with NHL was reported by De Vita et al using the combination chemotherapy, C-MOPP (cyclophosphamide, Oncovin, procarbazine, prednisolone)[29]. In 1976, the first of many reports appeared attesting to the efficacy of the Adriamycin-containing CHOP (cyclophosphamide, hydroxydaunomycin, Oncovin, prednisolone) regimen in intermediate- and high-grade lymphomas[30]. Since 1982, increasingly intensive third-generation drug regimens cooperating six or more drugs were developed (COP-BLAM[31] (cyclophosphamide, Oncovin, prednisolone, bleomycin, Adriamycin, Matulane), MACOP-B[32], ProMACE-CytaBOM[33] (prednisolone, doxorubucin, cyclophosphamide, and etoposide, followed by cytarabine, bleomycin, vincristine, and methotrexate with leucovorin rescue) in order to attain a more curative fraction of patients. However randomized investigation revealed no bettering of the CHOP results[34]. In the late 1980s, high-dose therapy in association with ABMT has become the treatment of choice for relapsed lymphomas[4, 35]. The discipline of treatment is currently employed earlier in the course of aggressive lymphomas.

To date, study of NHL by both laboratory and clinical discipline is highly intense and productive. The main reasons for this phenomenon can be summarised as follows:

1. The milestone discovery of epidemiologic characteristics of the disease leaded to the understanding of some aspects of the etiology of the disease, e.g., Burkitt's lymphoma in Africa, Mediteranean (α-heavy chain) lymphoma.

2. The strong evidence that viruses are associated with certain subtypes and may be etiologically important, e.g., Epstein-Barr virus (EBV), human T-cell leukemia/lymphoma virus (HTLV).

3. They can be considered as neoplasms of the immune system, and immunologic markers and methodologies can be applied to their study.

4. Many animal models exist that morphologically correspond to the human disease.

5. The neoplasms are highly responsive to therapy, which often is curative.

2.2 Epidemiology

2.2.1 Incidence

2.2.1.1 International Data

In general, age-adjusted incidence rates of NHL are higher in the more developed countries. The age-adjusted incidence rates for men varied from 3.7 in Poland to 14.0 per 100,000 person-years in U.S. from 1983 to 1987[36]. At the same time period, the age-adjusted incidence rates for men in Japan and Singapore were 4.1 and 6.0 per 100,000 person-years, respectively. The age adjusted incidence rates in 20 countries increased by about 50% or more within 2 decades. This phenomenon was observed in both men and women.

2.2.1.2 U.S. Data

The current U.S. age-adjusted rate for NHL is 15.1 per 100,000 person-years for both sexes combined. The incidence of NHL has been increasing much more rapidly than that of most other cancers[37]. Since 1970s, the annual age-adjusted incidence rates have been increasing at 3%

per year among women and 4% per year among men. Changes in the rates of diagnosis or diagnostic criteria do not account for the large increase over time. The reason underlying this dramatic disease increment is still unknown.

2.2.1.3 Thailand Data

The age-standardized incidence rate of NHL in Thailand is 2.8 per 100,000 person-years in male and 2.1 per 100,000 person-years in female from 1988 to 1991[38]. This data underestimate the true incidence as the case registry was derived mostly from the referral centers.

2.2.2 Age and Sex

Age greatly affects the risk of developing NHL. The incidence rate in the U.S. increases exponentially from 7.0 per 100,000 at age 30-34 years to almost 100 per 100,000 at age 80-84 years[37]. In Thailand, the incidence increases from 1.0 per 100,000 at age 20 to 10.0 per 100,000 at age 70-75 years[38]. The incidence in men is greater than women in almost all countries.

2.2.3 Risk Factors

2.2.3.1 Infectious Agents

2.2.3.1.1 Epstein-Barr Virus (EBV)

It is well recognized that EBV is associated with the endemic or classic Burkitt's lymphoma, reported mostly from Africa. It is essentially a pediatric disease, with a median age of diagnosis about 8 years. EBV genome has been identified in 100% of cases with chromosome 8 breakpoints generally within the *c-myc* oncogene[39]. In contrast, the less frequently so-called sporadic Burkitt's lymphoma which constituted a high proportion of Burkitt's

lymphoma outside Africa, is less likely to be involved with EBV (<15%) and has different genomic alterations[39].

Overall, EBV DNA has been shown in 10% to 30% of NHL tumors[40]. Seroepidemiologic studies show that elevated titers against viral capsid antigen preceding the diagnosis of NHL were associated with a twofold or threefold increased risk of developing NHL. This suggested the hypothesis that endogenous immunosuppression, prior to the development of NHL, leads to EBV reactivation. EBV may have a direct role in the development of a subset of NHL or it may be a surrogate marker for immune dysfunction.

2.2.3.1.2 Human Immunodeficiency Virus (HIV)

Since the first recognition of the significant increase in the frequency of NHL in acquired immunodeficiency syndrome (AIDS) in 1985, the definition of AIDS was changed to include the high-grade B-cell lymphoma[41]. A study by Beral and collegues among almost 100,000 unselected AIDS patients during 1981 to 1989 found that 3% of patients had NHL and that there was a 60-fold greater risk compared to the general population[42]. In a prospective study of a cohort of 116 patients with AIDS or symptomatic HIV infection treated with either zidovidine or dideoxyinosine and followed from 1985 through 1991, 12 patients developed lymphoma[43]. Overall, patients in this cohort had an 8% risk of NHL 2 years after initiation of antiviral therapy and 19% risk at 3 years. The incidence rate of developing NHL was 5.6% per patient-year, which is substantially higher than 0.015% in the general population. It is estimated that more than one-fourth of NHL cases occurring in the next decade may be secondary to the HIV infection.

The role of EBV in the pathogenesis of HIV-associated lymphomas continues to be debated. In a case-control study, Levine and coworkers demonstrated by in situ hybridization that EBV genome was present in 68% of HIV-positive lymphoma patients, compared with 15% of HIV negative

patients[39]. EBV early-region RNA however has been shown in 100% of primary CNS lymphoma tumors from patients with AIDS[44]. It is not clear whether the EBV is a reactivated carrier virus or important in the etiology. Although HIV does not appear to be causal in NHL, the immunosuppression related to HIV infection appears to be a major risk factor for NHL.

2.2.3.1.3 Human T-cell Lymphotropic Virus Type 1 (HTLV-1)

HTLV-1 is an RNA-containing C-type virus with low infectivity that accounts for a major fraction of lymphomas in endemic area, i.e., Kyushu, Japan, the Caribbean basin, the southeastern region of U.S. and Trinidad, Africa[45,46]. Overall, HTLV-1 carriers have a 2% to 5% lifetime risk of developing characteristic adult T-cell leukemia/lymphoma (ATL) as described by Uchiyama and collegues in the early 1970s[45,47]. Studies from Japan suggest that infection clusters in family. Vertical transmission through infected lymphocytes in brest milk, horizontal transmission through frequent sexual exposure over several years as well as transmission through blood donors had been documented[48]. Monoclonal integration of viral genome into tumor cells and the concordance of seroprevalence of HTLV and distribution of ATL strongly support the link between HTLV-1 and ATL.

2.2.3.1.4 Human Herpesvirus- 6 (HHV-6)

Detection of HHV-6 in NHL tissues has been infrequent[49,50]. A series from China reported HHV-6 genome by in situ hybridization in 18% of NHL tissues. Currently, the role of HHV-6 in NHL remains speculative.

2.2.3.2 Environmental Factors

2.2.3.2.1 Occupational Factors

A number of different occupations have been linked to NHL risk including anesthesiology, chemistry, construction, engineering, farming, fishing, forestry, leather work, mechanics, metal working, rubber working, vinyl chloride working and working in the food industry[51-56]. However most of these association are weak or inconsisitent. Exposure is a complicated issue in that it is difficult to pinpoint the effects of particles or solvents in a given occupational exposure.

2.2.3.2.2 Pesticides

Agricultural workers, who are in contact with a host of exposures, including pesticides, animal viruses, dusts, fuels, and oils, have been consistently noted to have increased risks for NHL and other hematologic malignancies[57]. Pesticides include herbicides, insectides, fungicides, and other agents[58]. Most studies of agricultural workers and other exposed populatiions showed an association between NHL and pesticide exposure; however, only some were significant. For example, 2,4-dichlorophenoxyacetic acid has been associated with twofold to eightfold risks of NHL in several studies[59-61]. The National Academy of Sciences in the U.S. had conducted a comprehensive review on the potential health effects of herbicides during 1992 to 1993. The report concluded that "evidence is sufficient that there is a positive association between exposure to herbicides and NHL"[62].

2.2.3.2.3 Hair Dyes

The constituents of hair dyes were found to be mutagenic by the in vitro assay in 1975[63]. Most of the epidemiological studies linking hair dyes and NHL have shown negative results. Cantor and co-workers, in a

population-based, case-control study, found that men hair dye users had a two-fold (95% CI: 1.3-3.0) increased risk of NHL[64]. Women hair dye users in another case-control study were estimated to have a relative risk of 1.5 (95% CI: 1.1-2.2)[65]. In addition, women who used permanent dyes or darker colored products appeared to have a greater risk. Among men, the risk was not significantly increased. Two other recent cohort studies showed no increased risk among permanent hair dye users. A subset analysis in one study found that women black hair dye users had a significant increased risk of fatal NHL[66]. Taken together, it is plausible that a subset of hair dye users, particularly those who use permanent dark-colored dyes over a prolonged period might have an increased risk for NHL.

2.2.3.2.4 Radiation

Ionizing radiation appears to have little or no role in the risk of NHL[67]. The role of ultraviolet exposure in the causation of NHL is highly speculative. A number of studies are underway to investigatie this hypothesis.

2.2.3.3 Primary and Secondary Immunosuppression

A clear association between genetic immunodeficiency syndromes, such as, ataxia-telangiectasia, common variable immunodeficiency, Wiskott-Aldrich syndrome and NHL has been documented. As many as 25% of patients with these disorders will develop cancers, and NHL accounts for more than 50% of such malignancies[68]. Similar to genetic immunodeficiency, immunosuppressive therapy in organ transplant recipients has also been reported to substantially increase the risk for NHL[69-71]. The risk can be as high as 46-fold, as compared with the general population[72]. It appears that the risk was higher among early transplant patients who received a transplant with poorer matches and more immunosuppressive medications. One hypothesis is that the development of lymphoma may be related to the chronic antigen stimulation of the graft combined with

immunosuppression[73]. Even in patients who received immunosuppression without grafts, there was an 11-fold increase for NHL.

2.3 Pathogenesis

2.3.1 *BCL-6* and Large Cell Lymphomas

The first clues to the pathogenesis of NHL which formed a critical lead for the identification of altered genes, derived from the observation of recurrent chromosome abnormalities. The most commonly detected cytogenetic aberrations in diffuse large cell lymphomas (DLCL) are shown in Table 1.

Cytogenetic analysis of large panels of NHL cases revealed relatively frequent chromosome alterations affecting band 3q27 in DLCL[74,75]. These observations suggested that 3q27 may be the site of a proto-oncogene whose structural alteration may be critical for DLCL pathogenesis. The cloning of chromosome breakpoints of several cases of t(3;14)(q27;q32) led to the identification of a genomic region involved in the majority of cases carrying 3q27 alterations called *bcl*-6[76]. *Bcl*-6 rearrangement are detectable in 40% of DLCL, significantly less frequently in follicular lymphoma (FL), and are not observed in other types of NHL or in other lymphoid malignancies[77]. The *bcl*-6 gene encodes a novel protein containing six zinc-finger motifs,

Table 1 Summary of recurrent chromosome abnormalities associated with DLCL

Chromosome	Frequency (%)	Gene
t(14;18)(q32;q21)	17	*bcl-2*
1q21-23	16	?
t(8;14)(q24;q32)	19	*c-myc*
+7	16	?
+12	13	?
3q27	12 (30)*	*bcl-6*
6q-	14 (20)*	?

*Frequency by molecular analysis

which have been shown to mediate specific DNA binding in a number of related transcription factors[78]. Functional studies have indicated that the *bcl*-6 protein can function as a transcription factor, which can bind a specific DNA sequence and repress transcription from adjacent promoters[79]. Thus, the physiologic function of the *bcl*-6 protein might be to repress the expression of genes carrying its specific DNA binding motif. Although trace amounts of *bcl*-6 protein are found in numerous tissue, high level are found in the B-cells restricted in the germinal centers in the lymph nodes (centroblasts and centrocytes), not in the immature B-cell in the bone marrow or mature progeny such as, plasma cells. It is conceivable that *bcl*-6 may be involved in the induction of germinal center-associated functions and that downregulation may be necessary for B-cells to progress further to memory cells or plasma cells.

In all cases displaying *bcl*-6 rearrangement, the 5' regulatory region containing the promoter sequences is either completely removed or truncated

leaving all of the coding region of *bcl*-6 gene are linked downstream to heterologous sequences which can originates from different chromosomes in different cases. The common functional consequence of these alterations is the juxtaposition of heterologous promoters to the *bcl*-6 coding domain, a mechanism called promoter substitution[80-82]. The finding that the normal *bcl*-6 promoter is substituted implies that *bcl*-6 expression is deregulated in these tumors, hence, prevent the differentiation of B-cells into plasma cells.

Among the heterogenous lymphomas in the DLCL spectrum, *bcl*-6 rearrangements are significantly more frequent in tumors with a pure diffuse large-cell histology which lack *bcl*-2 rearrangements. Considering DLCL can originate both *de novo* and from the 'transformation' of FL, and the latter typically carry *bcl*-2 rearrangements, this finding suggests that *bcl*-6 aberrations may be specifically involved in the pathogenesis of *de novo* DLCL.

2.3.2 *BCL-2* and Low Grade Lymphomas

Cytogenetic analysis has shown that a reciprocal translocation between chromosomes 14 and 18, t (14;18)(q32;q21), is common in follicular lymphoma (FL)[83]. This translocation results in the juxtaposition of the immunoglobulin heavy (IgH) chain joining segment (J_H) on chromosome 14 to a locus designated *bcl*-2 on chromosome 18 [84]. Cloning and sequencing of the cDNA for the *bcl*-2/IgH fusion transcript has demonstrated an open reading frame capable of encoding a 26 kD protein. Consequent to the translocation, the coding region of the *bcl*-2 gene is markedly overexpressed [85]. The *bcl*-2 gene product prevents programmed cell death (PCD), a term used interchangeably with apoptosis. Overexpression of *bcl*-2 has been shown to block cell death induced by a variety of stimuli[86,87].

The regulation of apoptosis is critical during normal B-cell ontogeny. As cells differentiate from pro-B to pre-B to sIg+ B-cells, there is extensive

loss as a result of PCD. In the pre-pro-B-cells, *bcl*-2 is highly expressed but is downregulated as cells differentiate[88,89]. Immunohistochemical technics in the hyperplastic lymph nodes demonstrates that *bcl*-2 expression in the germinal center (GC) is confined mainly to a small proportion of centrocytes of the follicular dendritic cells (FDC) in the apical light zone[90,91]. Based on the currently available information, it is clear that the pathogenesis of FL involves multiple steps. The tumor has its origin in the bone marrow, where the t (14;18) translocation occurs at the pro-B-cell stage of development. This event serves to enhance the survival of this particular clone which enters the circulation and eventually migrates to the lymph nodes. Cells from the t (14;18)- clone continue to develop on the B-cell pathway and are subject to antigenic selection which create a proliferative signal to the cell resulting in clonal expansion. The clone is distributed systematically to lymph nodes, bone marrow and home via integrin/adhesion molecule interaction. Overtime, additional genetic events occur, contributing to more aggressive clinical behavior and occasionally to frank transformation.

2.3.3 *C-MYC*, Epstein-Barr Virus and Burkitt's Lymphomas

The discovery of the nonrandom translocation involving chromosome 8 at band q-24, the location of the *c-myc* oncogene, associated with Burkitt's lymphoma and its 'variant' provided important insights into the pathogenesis of the disease[92,93]. The partner genes to *c-myc* are the immunoglobulin loci on chromososmes 14q32 (heavy chain, t (8;14)), 2p11 (kappa), or 22q11 (lambda). There is good evidence that the *c-myc* gene is transcriptionally deregulated in Burkitt's lymphoma and it is probable that transcriptional enhancers within the immunoglobulin regions -whether from heavy chain or light chain loci- which have juxtaposed to *c-myc* by the translocation, cause continuous transcription from *c-myc*[94]. The net consequence appears to be that the *c-myc* gene is regulated as if it were an immunoglobulin gene, i.e., it is constitutively expressed in these immunoglobulin-synthesizing cells.

However it is clearly understandable that *c-myc* dysregulation is not the only factor sufficient to induce neoplasia.

A number of paralells can now be drawn between the biology of EBV and EBV-associated Burkitt's lymphoma. It seems highly probable that the *c-myc*/immunoglobulin translocation occurs in a pro-B-cell at a low but regular frequency shortly before immunoglobulin rearrangement because of aberrant recombinase activity[95]. Infection with malaria, EBV and possibly other environmental infectious agents, would act on these cells via the polyclonal B-cell activation resulting in the expansion of these abnormal cells. Other necessary events such as mutation in the translocated *c-myc* gene occur, probably during the differentiation in the GC, resulting in malignant transformation. In some patients, additional p53 mutation occur and lead to tumor progression[96].

2.4 Pathologic Classification

With its introduction in 1956, the Rappaport classification of NHL became popular and widely used[26]. The approach was first to divide lymphomas by pattern, either nodular or diffuse, and then to subclassify them further on the basis of cytologic features (Table 2).

Although the Rappaport classification was popular with clinicians, scientific inaccuracies of the classification became apparent when the understanding of the immune system were expanding. New classifications, for example, the Lukes and Collins' classification, Kiel's classification and the World Health Organization classification were thus proposed to relate lymphoid neoplasms more closely to the normal immune counterpart[28,97,98]. The resulting situation was one of confusion and controversy. In an attempt to resolve these differences, the National Cancer Institute (NCI) funded an international study on 1,175 NHL cases treated in the same manner (Table 3). The classification was based purely on the histologic data and became

popular as the categories specified, namely, the low-, intermediate- and high-grade, are predictive of patients' survival[3].

Table 2 Modified Rappaport Classification

Nodular	
	Lymphocytic, poorly differentiated
	Mixed lyphocytic-histiocytic
	Histiocytic
Diffuse	
	Lymphocytic, well-differentiated
	Lymphocytic, intermediate differentiated
	Lymphocytic, poorly differentiated
	Mixed lyphocytic-histiocytic
	Histiocytic
	Undifferentiated, Burlkitt's type
	Undifferentiated, non-Burlkitt's type
	Lymphoblastic

Since the International Working Formulation was published in 1982, immunophenotypic and molecular analyses of hematolymphoid neoplasms have shown that the categories in the Working Formulation are immunologically and molecularly heterogeneous. Therefore, a group of pathologists known as the International Lymphoma Study Group has recently proposed a Revised European-American Classification of Lymphoid Neoplasms (REAL) in which morphologic, immunologic, and molecular data are incorporated into a provisional classification scheme (Table 4)[99]. Although the categories in the classification are not correlated clinically with prognosis, the new entities with distinctive immnunologic and molecular findings have breen included.

Table 3 International Working Formulation

Low grade

- A. Small lymphocytic, consistent with chronic lymphocytic leukemia
- B. Follicular, predominantly small cleaved cell
- C. Follicular, mixed small cleaved and large cell

Intermediate grade

- D. Follicular, predominantly large cell
- E. Diffuse, small cleaved cell
- F. Diffuse, mixed small and large cell
- G. Diffuse, large cell, cleaved or non-cleaved

High-grade

- H. Large cell, immunoblastic
- I. Lymphoblastic
- J. Small non-cleaved, Burkitt's or non-Burkitt's
- K. Miscellaneous
  - Composite
  - Mycosis fungoides
  - Histiocytic
  - Unclassifiable

Table 4 Revised European-American Classification of Lymphoid Neoplasms

B-cell Lymphoma

I. Precursor B-cell neoplasm: precursor B-lymphoblastic leukemia/lymphoma

II. Peripheral B-cell lymphoma

1. B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
2. Lymphoplasmacytoid lymphoma/immunocytoma
3. Mantle cell lymphoma
4. Follicular center lymphoma
5. Marginal zone B-cell lymphoma

   Extranodal (MALT type ± monocytoid B cells)

   Provisional subtype: nodal (± monocytoid)
6. Provisional entity: spleniic marginal zone lymphoma
7. Hairy cell leukemia
8. Plasmacytoma/myeloma
9. B-cell large cell lymphoma

   Subtype: primary mediastinal (thymic) B-cell lymphoma
10. Burkitt's lymphoma
11. Provisional entity: high-grade B-cell lymphoma, Burkitt's-like.

T-cell and Putative NK-cell Lymphoma

1. Precursor T-cell neoplasm: precursor T-lymphoblastic lymphoma/leukemia
2. Large granular lymphocytic leukemia

   T-cell type, NK-type
3. Mycosis fungoides/Sezary syndrome
4. Peripheral T-cell lymphomas, unspecified

   Provisional subtype: hepatosplenic ?? T-cell lymphoma

   Provisional subtype: subcutaneous T-cell lymphoma
5. Angioimmnoblastic lymphoma (AILD)

6. Angiocentric T-cell lymphoma
7. Intestinal T-cell lymphoma (± enteropathy-associated)
8. Adult T-cell lymphoma/leukemia
9. Anaplastic large cell lymphoma, CD 30+, T- and null-cell types
10. Provisional entity: anaplastic large cell lymphoma, Hodgkin's-like

In 1996, Intragumtornchai et al had reported the relative frequencies of pathologic subtypes of NHL in 1,391 patients diagnosed at six major medical institutes in Thailand based on the Working Formulation[1]. The result showed that the most common histologic subtype was the diffuse large cell-immunoblastic entity (39.9%). Follicular lymphomas constituted only 3.8% of the total cases. The immunophenotypic study revealed T-cell lymphoma in only 16.1% of the patients. This pattern of NHL characterized the feature of the disease in Asia, i.e., a low rate of follicular entity and a preponderance of the diffuse aggressive subtype. It is noteworthy that the incidence of T-cell NHL in Thailand is much less than the data reported from the Far East Asia (35%-45%)[100,101]. Table 5 shows the comparison of the distribution of NHL subtypes in various countries in Asia and U.S.

Table 5 Comparison of the relative frequencies (%) of various histologic subtypes in Asia and U.S.

Subtypes	Korea[102]	China[103]	India[104]	Thailand[1]	U.S.[3]
N	290	192	238	1391	1153
Low grade	5.1	12.5	12.2	12.8	33.7
Sm lymph	3.4	3.6	3.8	10	3.6
Fol sm cl	0.3	5.2	5.9	1.9	22.5
Fol mix	1.4	3.6	2.5	0.9	7.7
Int grade	72.2	39.1	+	++	37
Fol large	2.1	5.2	0.4	1	3.8
Dif sm cl	2.1	5.7	29.4	17.2	6.9
Dif mix	22.1	15.6	9.2	14.1	6.7
Dif large	45.9	12.5	+	++	19.7
High gr	21.7	43.2	+	++	17.2
Dif imm	12.4	12	+	++	7.9
Lympho	5.9	15.6	-	4.9	4.2
Sm noncl	3.4	15.6	5	4.6	5
Miscell	1	5.2	4.6	5.5	12.1

Diffuse large cell and immunoblastic subtypes totaled $^{+}$39.1% and $^{++}$39.9%

2.5 Clinical Presentation

Most patients with NHL come to medical attention because of the discovery of adenopathy. However, other problems such as abdominal fullness, pain in involved sites of disease, anemia, infection, neurologic symptoms or systemic symptoms such as fever, night sweats or weight loss can occasionally be the first sign of disease.

The clinical features of untreated patients are often typical for the individual histologic subtypes. For example, patients with follicular lymphomas most often present with painless adenopathy in the cervical, axillary, or inguinal regions. The nodes may have been present for years with fluctuation in size. Bone marrow involvement is present more frequently in follicular lymphomas (24%-50%) than in diffuse NHL (14%)[105]. Patients with small lymphocytic lymphomas present either with focal or generalized lymphadenopathy and often splenomegaly. Indeed, the disease is the tissue manifestation of chronic lymphocytic leukemia, i.e., the disease of the fifth decade with slow progression.[106]. The bone marrow is frequently involved and some patients showed monoclonal gammopathy. The clinical features of patients with diffuse aggressive NHL however are much more variable. Patients with diffuse large cell lymphoma (DLCL) often presents with relatively localized but rapidly progressive adenopathy. However patients often present with involvement of nonlymphatic tissues, such as, gastrointestinal tract, skin, breast, bone, skin, testes or central nervous system. Involvement of Waldeyer's ring is associated with an approximate 20% risk of gastrointestinal disease[107]. Bone marrow involvement at presentation is unusual found only in 10% of the patients. The most common presentation in patients with lymphoblastic lymphoma is mediastinal adenopathy[108]. Typically patients are male in their second or third decades. There is usually rapid dissemination of disease to the bone marrow and central nervous system. Pedriatric patients with Burkitt's lymphoma presents

with large extranodal tumors involving jaws and abdominal nodes. In contrast, sporadic cases outside Africa usually presents with large abdominal mass.

2.6 Diagnosis and Staging Procedures

Accurate histologic diagnosis is an essential first step toward proper care of patients with NHL. Adenopathy greater than 1 cm for a duration of 2-3 weeks in adult need tissue examination. Often in patients in whom lymphadenopathy is not present, invasive procedure has to be taken in order to arrive at proper diagnosis.

Once histologic diagnosis has been confirmed, various staging procedures must be done to direct therapy as well as to provide the prognostic information. Baseline studies that should be obtained in every patients include complete history and physical examination, complete blood count, liver chemistry, lactate dehydrogenase (LDH), renal function, chest x-ray, computed tomography (CT) of the abdomen, pelvis and chest (if the chest radiograph is suspicious) and bone marrow biopsy. Additional investigations such as cytologic examination of body fluid, gastrointestinal barium study, bone scan, magnetic resonance imaging (MRI) of the brain and spinal cord, etc, are performed as clinical indicated.

The results of these tests are utilized for designated patients based on the Ann Arbor Staging system which was developed for Hodgkin's disease (Table 6)[109]. After therapy has been initiated, previously positive tests should be repeated at appropiate intervals for evaluation of the effectiveness of the therapy.

Table 6 Ann Arbor Staging System

Stage I Involvement of a single lymph node region or of a single extranodal organ or site (I_E)

Stage II Involvement of two or more lymph node regions on the same side of the diagphragm, or localized involvement of an extranodal organ or site (II_E) and of one or more lymph node region on the same side of the diagphram

Stage III Involvement of lymph node regions on both sides of the diagphragm which may also be accompanied by localized involvement of an extranodal organ or site (III_E) or spleen (III_E) or both (III_{ES})

Stage IV Diffuse or disseminated involvement of one or more distant extranodal organs with or without associated lymph node involvement

Fever > 38^0C, night sweats, and/or weight loss > 10% of body weight during the preceding 6 months are defined as systemic symptoms and denoted by the suffix B. Asymptomatic patients are suffixed by the suffix A.

2.7 Natural Course and Prognostic Factors

NHL is a disease in which the outcome is known to be affected by a number of biologic parameters. Identification of patients with different long term prognoses could have important therapeutic implications. A widely accepted clinical model predicting outcome in patients with aggressive NHL known as "the international prognostic index" has been recently developed in 1993[5]. Sixteen single institutions and cooperative groups in the United States, Europe, and Canada participated in the study, known as the International Non-Hodgkin's Lymphoma Prognostic Factors Project. In this project, 3,273 patients who were treated with a doxorubicin-containing combination chemotherapy regimen were analysed. A group of 1,385 patients was randomly selected as a training sample in which to identify independent prognostic factors predictive for overall survival and relapse-free survival. The univariate associations between response and individual clinical features were analysed with Fisher's exact test for two-by-k tables then features independently associated with overall survival and relapse-free survival were identified in multivariate analysis by proportional-hazards regression. The five pretreatment characteristics that were independently significant adverse factors in the analysis were age > 60 years, tumor stage III or IV, number of extranodal involvement >1, ECOG performance status $\geq$ 2 and raised serum lactate dehydrogenase (LDH) level (Table 7). By assigning patients to one of four risk groups on the basis of the number of presenting risk factors, the predicted five-year survival rates in the four risk groups identified (low, low-intermediate, high-intermediate and high) were 73%, 51%, 43% and 36%, respectively. Of the total patients, the high-intermediate and high-risk cases together comprised 38% of all patients. For patients aged $\leq$ 60 years, the clinical features that were independently associated with survival included stage, LDH and performance status. An age-adjusted model based on these three features identified four risk groups of patients (low, low-intermediate, high-intermediate and high) with predicted five-year survivals of 83%, 69%, 46% and 32%. Increased risk of death was due to a lower rate of complete

remission and a higher rate of relapse from a complete response. Based on these two indexes, patients identified as poor-risk category should therefore be considered as candidates for a more effective experimental therapy.

Recently a number of studies had shown that the international prognostic index invented for patients with aggressive lymphomas could also be used precisely to predict prognosis in NHL patients with all malignancy grades[110,111]

Table 7 Factors independently prognostic of overall survival in the training sample

Factor	Relative risk	P-value
All patients (n=1385)		
Age (≤ 60 vs > 60)	1.96	< 0.001
Serum LDH (≤1 x normal vs >1 x normal)	1.85	< 0.001
Performance status (0 or 1 vs 2-4)	1.80	< 0.001
Stage (I or II vs III or IV)	1.47	< 0.001
Extranodal involvement (≤1 site vs >1 site)	1.48	< 0.001
Patients ≤ 60 years old (n=885)		
Stage (I or II vs III or IV)	2.17	< 0.001
Serum LDH (≤1 x normal vs >1 x normal)	1.95	< 0.001
Performance status (0 or 1 vs 2-4)	1.81	< 0.001

2.8 Treatment of patients with aggressive NHL

2.8.1 Conventional Therapy

Under the Working Formulation, aggressive NHL include diffuse mixed (DM), diffuse large-cell (DLC) and diffuse large-cell immunoblastic lymphoma (DLCI)(category F, G, H). Patients with localized stage I or minimal stage II disease can be treated with a less aggressive approach than those with more extensive disease but still with curative intent. Involved field radiation has been found to induce up to 80% disease-free survival rate in one study with extensively staged patients[112]. Several studies have now reported improvement with the addition of short-course combination chemotherapy before involved field radiation[113,114]. A study conducted at Vancouver evaluated 78 patients with stage I, or limited stage II DM, DLC and DLCI lymphomas who had no B symptoms and had tumors < 10 cm in diameter [113]. The patients received 3 courses of CHOP chemotherapy followed by involved-field radiation to the original site of disease equivalent to 3,000 cGY in 10 fractions. The complete remission rate was 99%. At a median follow-up time of 30 months, the actuarial disease-free and overall survival were 84% and 85%, respectively.

Whereas about 30% of patients with aggressive lymphomas have localized disease at diagnosis, most have extensive disease and need more extended treatment. Many combination chemotherapy programs have been evaluated for the treatment of these patients (Table 8).

Table 8 Frontline regimens for treatment of patients with aggressive NHL

Regimen	No.patients	% CR	% Survival	Median follow-up	Ref.
CHOP	418	53	30	13.4 yr	115
M-BACOD	121	71	50	68 mo	116
MACOP-B	125	84	69	18 mo	32
Promace-MOPP	79	74	65	4 yr	117
ProMACE-CytaBOM	78	65	57	36 mo	118
COP-BLAM	51	84	65	2 yr	119

The first success chemotherapy regimen was CHOP invented by the Southwest Oncology Group (SWOG) in the middle 1970s[30]. It consists of vincristine, cyclophosphamide, hydroxyldaunomycin and prednisolone administered every 21-28 days for a total of 8 courses. The regimen was fairly easy to administer and has gained widely acceptance. When administered to patients with advanced stage aggressive NHLs, complete remission is obtained in 45-55 percent and overall 5-year disease-free survival is achieved in 30-35 percent of the patients[4]. In the 1980s, the so-called second and third-generation regimens added differerent drugs or utilized different methods of administration, such as ProMACE-CytaBOM, m-BACOD (methotrexate with leucovorin rescue, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone) and MACOP-B, were developed by several large lymphoma-referral centers[32,117,118]. The methotrexate was added in an attempt to increase the central nervous system penetration. Others also created COP-BLAM, a six drug regimen, based on the existing BACOP (bleomycin, Adriamycin, cyclophosphamide, Oncovin, prednisolone) regimen[119]. Most of these newer regimens demonstrated increased rates of complete remission of 65-85% and survival

rates of 50-65%. However, most of these studies are reported from single institutions, therefore, likely to contain some centripetal bias. In addition, the study populations are not homogeneous among studies and the results were compared with historical controls. To compare therapeutic outcome among these chemotherapy regimens, a recent prospective, randomized, controlled trial comparing CHOP with m-BACOD, ProMACE-CytaBOM and MACOP-B, was conducted in 1993 by Fisher and collegues[34]. The outcomes of interest were tumor response rate, toxicities and long-term survival. It was a multicenter study comprising a total of 1,138 patients. The rate of complete response was 44% for CHOP (n=225), 48% for m-BACOD (n=223), 56% for ProMACE-CytaBOM (n=233) and 51% for MACOP-B (n=218) ($p>0.05$). The rate of patients alive without disease at three years was estimated as 41% in patients who received CHOP and MACOP-B and 46% in the m-BACOD and ProMACE-CytaBOM group ($p > 0.05$). However, there was a significant increase in the incidence of fatal and life-threatening toxicities in the m-BACOD and MACOP-B groups compared to CHOP ($p = 0.001$). According to the tables displayed by Sackett[120], the study has a big enough sample size to show a relative risk reduction of treatment-failure rate of > 25% should it have occurred. From the standpoint of this randomized controlled trial, CHOP remains the standard front-line chemotherapy for patients with aggressive NHL. It is however noteworthy to emphasize that with the currently available doxorubicin-based chemotherapy, the predicted five-year survival rate of patients with low-, low-intermediate, high-intermediate and high-grade according to the international index were 83%, 69%, 46% and 32%, respectively[5]. Based on these data, patients identified as poor-risk category (high-intermediate and high-risk cases) are therefore the candidates for a more effective experimental therapy.

2.8.2 High-dose Therapy and Hematopoietic Stem Cell Transplantation

Meyer and coworkers had recently conducted a meta-analysis of 14 randomized trials and a cross-trial analysis of 22 comparative trials using a weighted least squares linear regression to determine whether dose intensity of chemotherapeutic regimens correlates with the complete remission rate in adult patients with advanced stage intermediate and high-grade lymphoma[6]. Reports were identified using MEDLINE, references from review articles and through review of selected abstracts. The meta-analysis showed a relative probability of achieving complete remission of 1.34 (95% confidence interval, 1.13 to 1.58) favoring the pooled arm of high dose intensity. Cross-trial analysis showed a relatively weak association between dose intensity and remission rate ($r = 0.49$, $p = 0.0001$). In addition, two of four reports retrospectively assessing received dose intensity suggested that increased dose intensity is associated with superior remission rates. The study suggested that dose intensity may correlate with remission rate in the patients and prospective properly designed trials directly testing dose intensity are needed to confirm the hypothesis.

The first lymphoma patient population that high-dose therapy and hematopoietic stem cell transplantation was applied to were patients with relapsed aggressive lymphomas. Early non-randomized trials consistently demonstrated long-term disease-free-survival (DFS) in 20-50% of these patients[121-123]. Compared to results of 5-10% long-term DFS in similar patients receiving conventional salvage chemotherapy, this result appeared to represent an improvement[124,125]. Colombat et al had reported the result of ABMT in 46 adult NHL patients with heterogeneous clinical response between 1978 and 1988[14]. Twelve patients were in first complete remission (CR), 5 in first partial remission (PR) to first-line chemotherapy, 21 in chemosensitive relapse and 8 were in progressive disease

(chemoresistance). The 3 year disease-free survival was 60% for all patients, 0% for refractory disease, 82% for patients in first complete remission (CR) and partial remission (PR) and 60% for patients with sensitive relapse. Three patients died of neutropenia-related sepsis. The results suggested that high dose therapy with ABMT in chemosensitive NHL had a good results and should be administered in first CR. Another study was conducted as a multicenter randomized controlled trial compared patients with chemotherapy-sensitive, relapse who received an additional four cycles of salvage DHAP (dexamethasone, high-dose cytarabibe, and cisplatin) therapy with patients who received high-dose therapy followed by ABMT[126]. The patients in the transplant arm had a superior event-free survival (EFS, 46% vs. 12% at 5 years, p=0.001) and OS (53% vs. 32% at 5 years, p=0.04) compared to those patients receiving conventional salvage therapy. Despite the clear indication for transplantation in patients with relapsed aggressive NHL, there are still controversy whether to administer the procedure in patients in first CR.

Several studies have now explored the use of dose-intensive chemotherapy and autologous transplantation in NHL patients in first partial or complete remission. An Italian study randomized patients in first partial remission after receiving a doxorubicin-based regimen to high-dose chemotherapy and autologous transplant or DHAP salvage chemotherapy [121]. The patients in the transplant arm had a trend towards improvement in EFS (73% vs. 52%) and OS (73% vs. 59%) after 55 months. However, a Dutch Hovon Study of 69 patients in first partial response after 3 cycles of CHOP chemotherapy randomized to ABMT following cyclophosphamide and total body irradiation (TBI) or continue with 5 more cycles of CHOP demonstrated a trend towards superior EFS (53% vs. 41% at 4 years) and OS (85% vs. 56% at 4 years) for the group treated with chemotherapy alone [128]. It is noteworthy that these two studies enrolled patients with all prognostic grades which might dilute the effect of high-dose therapy in the high-risk patients.

Several studies have now explored the role of dose-intensive chemotherapy and autologous transplant in high-risk patients in first complete or partial remission. In a study by Vitolo et al, 50 patients with high-risk DLC lymphomas were given an induction with 8 weeks of MACOP-B, intensified with a 3-day course of mitoxanthrone 8 mg/m^2 with high-dose cytarabine 2 gm/m^2 every 12 hours and dexamethasone (MAD) followed by BEAM (BCNU, etoposide, Ara-C, and Melphalan) conditioning regimen and peripheral stem cell harvesting[129]. With a median follow-up of 32 months, the OS is 56% and the failure-free-survival (FFS) is 50%. These results are improved over what would be expected in a high-risk population receiving standard anthracycline therapy. In one of the first published randomized studies by Haioun et al, all risk groups of patients with advanced aggressive NHL received the LNH-84 protocol[130]. If they achieved a CR, they were randomized to a consolidation regimen (N = 234) or CBV followed by ABMT (N = 230). The EFS in the consolidation group was 52% at 3 years versus 59% in the transplant group (p = 0.46). However, in the high-intermediate and the high-risk groups, the 4-year EFS in the consolidation group was 39% compared with the transplant group of 59% (p = 0.02). Recently, Gianni et al had reported encouraging results of up-front dose-intensive with stem cell support in 98 newly diagnosed high-risk patients with DLC and DM lymphomas[22]. Patients were randomized to receive either MACOP-B chemotherapy or to a high-dose sequential chemotherapy regimen with autologous peripheral blood progenitor support. The EFS (76% vs. 49%, p = 0.004) and OS (81% vs. 55%, p = 0.09) were both superior in the high-dose sequential therapy group. In view of these results, further evaluation of the concept of transplant in first complete remission for aggressive NHL in the high-intermediate and the high-risk patients is warranted.

2.8.3 Techniques and Utilities of Peripheral Blood Progenitor Cell Transplantation (PBPCT)

First introduced into clinical arena in the mid 1970s, transplantation of PBPC is now one of the most rapidly evolving fields in clinical medicine. PBPCT has currently replaced bone marrow as the major source of hematopoietic stem cell autografting for patients receiving high-dose chemoradiotherapy. This trend is due, in part, to the perception that multiple leukapheresis procedures are less morbid than a marrow harvest. Most importantly, several randomized studies have demonstrated faster granulocyte and platelet recovery post-transplant compared to patients who received bone marrow alone which might lead to diminution of antibiotics and platelet usage as well as faster hospital discharge, hence, the overall cost-utility of the treatment.

At steady state, hematopoietic stem cells are present only at low concentrations in peripheral blood. However, following cytotoxic therapy, the quantity of early hematopoietic stem cells is dramatically increased during the granulocyte recover period[131]. The principle of this observation have been designed and used as the "stem cell mobilization" in patients scheduled for the high-dose therapy. The most frequently used chemotherapy for this purpose were cyclophosphamide, etoposide or the drug combination[132]. The administration of recombinant growth factors, such as, G-CSF, GM-CSF, has a similar effect, and the peak of stem cell yield is around 5 to 6 days after the initiation of the growth factors[19,133]. However, the highest yield of PBPC is obtained after mobilization with the combination of growth factors and chemotherapy[134].

Following mobilization, PBPC are harvested by leukapheresis. The goal is to harvest 2-5 x 10^6 CD34+ cells or 20 x 10^4 granulocyte-macrophage colony-forming unit (CFU-GM) of recipient weight[135]. Cells obtained by

leukapheresis are cryopreserved (-196^{0}C with DMSO or -120^{0}C with 6% HES and DMSO) until all collections are completed. Following the conditioning regimen, cells are thawed and infused rapidly to the patients.