CLINICAL OUTCOMES OF INDUCTION THERAPY WITH ADDITION OF HIGH DOSE METHOTREXATE AND CYTARABINE TO EXISTING STANDARD TREATMENT IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSED LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

aaIPI	Age-adjusted International Prognostic Index
ABC	Activated B-cell like
AIDS	Acquired immune deficiency syndrome
ALC/AMC	Absolute lymphocytes: Absolute monocytes
ALDH	Aldehyde dehydrogenase
ALL	Acute lymphocytic leukemia
ANC	Absolute neutrophil count
AraC	Cytarabine
aRDI	Average relative dose intensity
BBB	Blood brain barrier
BCL2	B-cell lymphoma 2
BCNU	Carmustine (bis-chloroethylnitrosourea)
CD	Castleman disease
CNS	Central nervous system
CR	Complete remission
CRR	Complete response rate
CT	Computerized tomography
Delta -SUV	Delta-Standardized uptake value
DFS	Disease-free survival
DHFR	Dihydrofolate reductase
DLBCL	Diffused Large B-Cell Lymphoma
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
ECG	Echocardiography
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free survival
EOT	End of treatment
GC	Germinal-centre like
G-CSF	Granulocyte colony-stimulating factor
GEP	Gene expression profiling
HAART	Highly active antiretroviral therapy
HBV	Hepatitis-B virus
HCV	Hepatitis-C virus
HDC/ASCT	High dose chemotherapy/Autologous stem cell transplant
HIV	Human immunodeficiency virus
IgM	Immunoglobulin M
IPI	International Prognostic Index
IT	Intrathecal
IWC	International Workshop Criteria

LDH	Lactate dehydrogenase
MOH	Ministry of Health
MREC	Medical Research Ethics Committee
MTX	Methotrexate
NHL	Non-Hodgkin Lymphoma
NMRR	National Medical Research Register
NOS	Not otherwise specified
ORR	Overall response rate
OS	Overall survival
PBL	Plasmablastic lymphoma
PD	Progression disease
PEL	Pleural effusion lymphoma
PET	Positron emission tomography
PFS	Progression free survival
PR	Partial remission
PTLD	Post-transplant lymphoproliferative disorder
R-CHOP	Rituximab-Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone
REAL	Revised European American Lymphoma Classification
RNA	Ribonucleic acid
SD	Stable disease
SLE	Systemic lupus erythematosus
SPSS	Statistical Package for Social Sciences
TBI	Total body irradiation
TLS	Tumour lysis syndrome
TRM	Treatment related mortality
WBC	White blood count
WHO	World Health Organization

LIST OF APPENDICES

HASIL KLINIKAL TERAPI INDUKSI SELEPAS PENAMBAHAN DOS TINGGI METHOTREXATE DAN CYTARABINE KEPADA REGIMEN STANDARD DALAM KALANGAN PESAKIT BARU DIFFUSED LARGE B-CELL LYMPHOMA: ANALISIS RETROSPEKTIF

ABSTRAK

Diffused large B-cell lymphoma (DLBCL) merupakan penyakit yang heterogenus di mana hasil rawatan bergantung kepada ciri-ciri klinikal dan biologikal penyakit. Walau bagaimanapun, rawatan induksi optimum untuk pesakit DLBCL yang berisiko tinggi masih tidak jelas dan memerlukan kajian klinikal yang lebih untuk memahami dan menilai kaedah alternatif. Kajian ini bertujuan untuk menilai kadar respon dan *remission* jangka masa panjang hasil rawatan induksi selepas penambahan dos tinggi methotrexate dan cytarabine kepada regimen RCHOP dalam kalangan pesakit yang didiagnos dengan DLBCL. Sebanyak 62 pesakit DLBCL yang didiagnos antara bulan Januari 2011 sehingga Disember 2014 telah dianalisa secara retrospektif. Purata umur sewaktu diagnosis untuk pesakit DLBCL adalah 50 ± 13.4 tahun (julat 18 – 77 tahun). Seramai 38 pesakit menerima rawatan RCHOP/CHOP bersama methotrexate atau cytarabine atau kedua-duanya (RCHOP/CHOP+MA/M/A) sementara 24 pesakit hanya menerima RCHOP/CHOP regimen. Kaedah Kaplan-Meier dan ujian log-rank telah digunakan untuk menganalisis faktor-faktor bagi analisis univariate. Kadar *complete remission* (CR) adalah lebih tinggi bagi pesakit yang menerima rawatan RCHOP/CHOP+MA/M/A (65.8%) berbanding dengan pesakit yang menerima rawatan

RCHOP/CHOP (37.5%). Terdapat perbezaan yang signifikan dari segi *overall survival* (OS) bagi pesakit yang menunjukkan simptom B berbanding dengan pesakit yang tidak menunjukkan simptom B ($p = 0.02$). Berdasarkan kepada respon awal rawatan, pesakit yang mencapai *complete remission* (CR) didapati memaparkan peningkatkan OS yang signifikan ($p < 0.0001$). Antara 62 pesakit DLBCL yang menerima rawatan kemoterapi $\geqslant$ 4 kitaran, 2 pesakit (0.5%) meninggal dunia disebabkan komplikasi rawatan (TRM). Dalam kajian ini, median *overall survival* (OS) dan median *progression free survival* (PFS) masih belum dicapai. Median jangkamasa pemonitoran pesakit (*follow-up*) adalah 20 bulan (julat 2-43 months). OS pesakit untuk 2 tahun adalah sebanyak 74% dengan 2 tahun PFS mencecah 71%. Kadar OS dan PFS bagi 38 pesakit yang menerima rawatan RCHOP/CHOP+MA/M/A menunujukkan perbezaan yang ketara berbanding dengan kumpulan pesakit yang menerima rawatan RCHOP/CHOP ($n = 24$) dengan nilai $p = 0.03$ and $p = 0.04$, masing-masing. Selain itu, kumpulan perempuan yang terlibat dalam kajian ini menunjukkan pola yang lebih baik dari segi hasil klinikal. Secara kesimpulannya, penambahan methotrexate dan cytarabine kepada regimen RCHOP/CHOP mendatangkan manfaat dari segi 2 tahun OS dan PFS untuk pesakit DLBCL serta mengukuhkan lagi data kajian dari negara lain.

CLINICAL OUTCOMES OF INDUCTION THERAPY WITH ADDITION OF HIGH DOSE METHOTREXATE AND CYTARABINE TO EXISTING STANDARD TREATMENT IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSED LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY

ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is a heterogenous group of diseases with various outcomes depending on the clinical and biological features. Optimal induction therapy for high-risk DLBCL is still unclear, and clinical trials are needed to evaluate alternative approaches. This study aimed to evaluate the response rates and long term remission of induction therapy with addition of high dose methotrexate and cytarabine onto RCHOP for patients diagnosed with DLBCL. 62 DLBCL patients diagnosed between January 2011 to December 2014 were retrospectively analyzed. Mean age at diagnosis for enrolled patients was 50 ± 13.4 years (range 18 – 77 years). A total of 38 patients received RCHOP/CHOP plus methotrexate or cytarabine or both (RCHOP/CHOP+MA/M/A) while 24 patients received only RCHOP/CHOP regimen. Kaplan-Meier method and log-rank test were used to analyze factors for univariate analysis. The complete remission (CR) rate was higher in the RCHOP/CHOP+MA/M/A arm (65.8%) compared to the RCHOP/CHOP arm (37.5%). There was a significant difference in overall survival (OS) comparing patients manifested with B symptoms and patients without B symptoms ($p = 0.02$). With respect to the initial response of frontline therapy, patients who achieved a complete remission (CR) was associated with

significantly improved OS ($p < 0.0001$). Among 62 DLBCL patients who received $\geqslant 4$ cycles chemotherapy, 2 (0.5%) experienced treatment-related mortality (TRM). The median overall survival (OS) and median progression free survival (PFS) are not reached. Median duration of follow-up was 20 months (range 2-43 months). The 2-year OS was 74% with 2-year PFS 71%. Overall survival and progression free survival rates of 38 patients received RCHOP/CHOP+MA/M/A showed significantly better compare to RCHOP/CHOP group ($n = 24$) with $p = 0.03$ and $p = 0.04$, respectively. Besides, there is a trend of better outcome in female cohort. In conclusion, addition of methotrexate and cytarabine onto RCHOP/CHOP regimen resulted in improved 2-year OS and PFS of DLBCL patients which confirms the results of previous published study in other countries.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

The most common subtype of non-Hodgkin lymphoma (NHL) is the Diffuse large B cell lymphoma (DLBCL) which accounting for approximately one third of all newly diagnosed lymphoid malignancy case (Martelli et al., 2013, Sehn and Gascoyne, 2014). According to the Malaysia National Cancer Registry Report 2007-2011, lymphoma cases accounted for 4.3% of all malignancy incidence reported (National Cancer Registry, 2011). Besides, the Melaka Cancer Registry 2015 indicated that the prevalence of lymphoma cases is 10: 100,000 population and DLBCL is 5:100,000 (Sabtu, 2015). This group of disease is derived from large neoplastic B cells and clinically manifest aggressive presentation (Savage, 2006). Based on the clinical signs and symptoms and morphology characteristics, variants and subtypes of DLBCL have been identified in the World Health Organization (WHO) classification and Real European-American Lymphoma (REAL) (Harris et al., 1994, Swerdlow et al., 2008). Even though morphological, biological and clinical studies are able to differentiate DLBCL into distinct disease entities, there is no clear description and accepted criteria for sub-classification in many cases, which still remain them biologically and clinically heterogenous. By and large, these are called as DLBCL, not otherwise specified (NOS) (Swerdlow et al., 2008).

As the age increases; reaching a peak in the late 70's years old and above, the incidence of DLBCL increases as well. Conversely, other types of aggressive NHL for example primary mediastinal lymphoma and Burkitt lymphoma, occur at a lower median age (Peyrade et al., 2011). Differences should identified between cases arising de novo (referred as primary) and those categorized under progression or transformation (referred to secondary) of a less aggressive lymphoma, which include follicular lymphoma, marginal zone lymphoma, nodal lymphocyte predominant Hodgkin lymphoma, or chronic lymphocytic leukemia/small lymphocytic lymphoma, as most of the causes of DLBCL remain unknown.

Factors such as exposure to agent that produces peculiar molecular as well as acquired immunodeficiency and congenital states may be predisposing to the disease (Hartge P, 2004). Study done by De Roos and colleagues shown that risk of NHL increases with the use of several pesticides (De Roos et al., 2003). Drugs used in treatment of cancer such as alkylating agents significantly increases the occurrence of lymphomas as secondary tumour when used together with ionizing radiation, particularly when high volumes of bone marrow or spleen are irradiated (Martelli et al., 2013). More incidence of diffuse aggressive lymphomas which involved the brain were seen in patients taking immunosuppressive drugs, especially after organ transplants (Swerdlow et al., 2008). In fact, DLBCL were also found in patients with inherited immunologic deficiency diseases, and in families of patients with immunologic disorders (Vianna, 1977).

Over the last decade, relationships between DLBCL and viruses have become a debatable issue. There are few studies reported that aggressive lymphomas were associated with hepatitis-C virus (HCV) infection (De Vita et al., 1997, Ascoli et al., 1998). Additionally, there are a few DLBCL cases reported of EBV-positive while patients receiving Alemtuzumab treatment with no prior history of haematological malignancy (Abruzzo et al., 2002, Ghobrial IM et al., 2003, Roch et al., 2008, Sohani et al., 2010). The rise of several types of lymphoma has been linked with human immunodeficiency virus (HIV) infection as well (Diebold et al., 1997). Normally these arises in an early phase of AIDS evolution, which usually were quite aggressive and diffused tumours. Nevertheless, the emergence of highly active antiretroviral therapy (HAART) treatment has significantly improved and reduced the number of lymphoma among HIV positive patients. Besides, the tolerability of chemotherapy also increased among the HIV positive patients (Antinori et al., 2001, Ratner et al., 2001, Hoffmann et al., 2003, Rossi et al., 2010).

DLBCL is diagnose with a biopsy examination of affected tissue preferable excisional biopsy to make a proper diagnosis. Incisional biopsy or core needle biopsy is permitted only in case when excision is not possible (Andersson et al., 1992). Evaluation before treatment and staging are the next critical step once the diagnosis established. Ann Arbor staging system with Cotswolds modification is the standard staging used to stratify DLBCL (Carbone et al., 1971, Lister et al., 1989). Developed in 1971, Ann Arbor staging system principally based upon the use of staging laparotomy and lymphangiogram. It has a four stages; ranging from stage I to stage IV (Carbone et al., 1971).

However, this has been replaced with a bone marrow trephine biopsy and a CT scan. Subsequently in year 2014, further revisions to clarify the role of positron emission tomography (PET) and improvised extranodal involvement definition were suggested in Lugano classification (Barrington et al., 2014, Cheson et al., 2014). Cases of NHL are usually categorized as early stage disease (stage I to II) or advanced stage disease (stage III to IV). Further stratification into favorable and unfavorable subsets were done for patients with early stage NHL.

Prognostic factor for DLBCL can be divided into four, mainly those related primarily to the patient clinical characteristic (age and performance status), those related to aggressiveness parameters (β2-microglobulin level and serum LDH level), those related to therapeutic option and those related to tumour or disease itself (stage, proliferating, extranodal involvement and tumour burden). Presence of the lymphoma in the extranodal sites, for examples, brain (Ferreri, 2011) and testis (Zucca et al., 2003) need special strategies and it usually associated with poor prognosis. Achieving a good response rate post induction treatment is pivotal and able to predict the treatment outcome.

Later, in regard to more than 4000 patients' clinical factors, the *International Prognostic Index (IPI)* and *age-adjusted International Prognostic Index (*aaIPI*)* was established and used as an outcomes predictors (Project, 1993). In fact, it has been proven to be better in term of predicting survival outcome compared to the Ann Arbor classification. In the clinical practice especially stratifying patients, parameters such as aaIPI, which involves LDH level, patient's performance status and disease stage, are

frequently used. During post-rituximab era, an updated version has been created (R-IPI) (Sehn et al., 2007). Although robustness of IPI has been reported in different series of DLBCL, it still represents the only benchmark to prognosticate DLBCL, even in the rituximab era (Ziepert et al., 2010).

There are a vast number of biological parameters which have been verified as factors to predict DLBCL prognosis, creating a research field for understanding the disease in depth as well as designing new therapeutic options for better survival. In fact, biological heterogeneity in DLBCL can be categorized into two distinct groups based on their postulated normal lymphoid cell counterpart; activated B-cell like (ABC) and germinal-centre like (GC) DLBCL (Lenz et al., 2008). The GC DLBCL shown excellent treatment outcome with standard R-CHOP therapy, whereas the ABC DLBCL is associated with poorer outcome. Hernandez and colleagues reported that the use of lenalidomide in salvage therapy is associated with a significant higher response rate in ABC-DLBCL as compared to GCB-cell-DLBCL (Hernandez-Ilizaliturri et al., 2011). This shown that more promising studies are needed particularly focusing on gene expression profiling (GEP) to be practise in the clinical setting.

Despite being an aggressive disease, DLBCL can be potentially curable malignancies either with combine modality therapy or chemotherapy alone (Martelli et al., 2013). The patients with limited disease seem to have better cure rate with 80-85% of 5-year progression free survival (PFS) as compared to the advanced disease or symptomatic disease, which is 50% of 5-year PFS (Project, 1993).

Individual IPI score and age have an influence on the option for first line treatment (induction) for DLBCL patients. The CHOP-21 regimen (combination of cyclophosphamide 750 mg/m^2, doxorubicin [Adriamycin] 50 mg/m^2, vincristine [Oncovin] 1.4 mg/m^2 [to a maximum of 2 mg] given on day 1 and tablet prednisolone 60 mg/m^2 daily from Day 1- Day 5 plus Rituximab) remain the standard treatment for patient with DLBCL. This combination of chemotherapy has made a significant breakthrough transforming the fatality of patient to often curable disease. Even though outcome of CHOP-14 treatment shown to be better than CHOP-21 in elderly patients (> 60 years), incorporating rituximab to CHOP (R-CHOP) in either 14- or 21- day intervals shown comparable efficacy. In fact, patients received R-CHOP-14 shown to have higher toxicities level (Coiffier et al., 2002, Pfreundschuh et al., 2006b, Cunningham et al., 2011). Nevertheless, the clinical outcome still remains poor when treating the high-risk DLBCL (IPI score $\geq$ 3), with an overall survival (OS) rate of 55% a 4-year progression-free survival (PFS) rate of 53% (Sehn et al., 2007).

In order to overcome these poor results among young patients with high risk features, up-front stem cell transplant was suggested as consolidation after R-CHOP. However, up-front consolidation needs transplant specific procedures and it has many associated toxicities. Besides, its actual benefits remain controversial (Stiff et al., 2011). A higher intensity novel regimen consisting of rituximab plus hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with rituximab, high dose methotrexate and cytarabine (R-HCVAD/R-MA) has a significant positive result in mantle cell lymphoma (Romaguera et al., 2005, Bernstein et al., 2013),

acute lymphoblastic lymphoma (Thomas et al., 2006, Thomas et al., 2010), and Burkitt lymphoma (Thomas et al., 2006). Additionally, a retrospective analysis done by Fayad shown that 27 consecutive treated DLBCL patients age ≤ 60 years old in MD Anderson Cancer Centre had a 95% complete response rate and a 3-year PFS rate of 86% (Fayad et al., 2007).

1.2 Rationale for this study

Currently, optimal induction chemotherapy regimen for high risk DLBCL is remain questionable, and more studies are necessary to assess alternative approaches other than R-CHOP. Studies of different intensive chemotherapy regimen in the US and UK have positive impact on overall survival rate. However, there is no study on alternative induction chemotherapy conducted in Malaysia and R-CHOP21 regimen remains standard despite poor outcome in certain high risks patients. In addition, most of the studies conducted in overseas were not representative of Asian population. They can only act as a guide and might leads to variations in the responses in different population and ethnicity. In fact, study shown that there are inter-ethnic differences in drugs effects or their influence by ethnic-specific gene variants. Therefore, is crucial to evaluate the effect of alternative induction chemotherapy focusing on high risk DLBCL.

The aim of this study was to evaluate the clinical outcomes of induction therapy with addition of high dose methotrexate and cytarabine onto RCHOP21 for patients diagnosed with DLBCL, in terms of response rates and long-term remission. Besides,

this investigation will provide insight to the field of haematology in Malaysia, through providing recommendations to improve the standard of care for DLBCL patients.

1.3 Objectives of this Study

1.3.1 Primary Objective

To evaluate the complete response rate of induction therapy with addition of high-dose Methotrexate and/or Cytarabine onto standard R-CHOP/CHOP (R-CHOP/CHOP+MA/M/A regimen) as compare to standard R-CHOP/CHOP regimen for patients with newly diagnosed Diffuse Large B-Cell Lymphoma (DLBCL) in haematology ward, Hospital Melaka, Malaysia.

1.3.2 Secondary Objectives

1) To assess the response rate (complete response, partial response, stable disease or disease progression) at mid-cycle and end of treatment among patients who newly diagnosed with DLBCL treated with standard R-CHOP/CHOP alone as compared to R-CHOP/CHOP+MA/M/A regimen.
2) To explore the treatment related mortality among patients who newly diagnosed with DLBCL treated with standard R-CHOP/CHOP alone or R-CHOP/CHOP+MA/M/A regimen.
3) To assess the overall survival (OS) and progression free survival (PFS) rate among patients who newly diagnosed with DLBCL treated with standard R-CHOP/CHOP alone as compared to R-CHOP/CHOP+MA/M/A regimen.

4) To evaluate correlation between age, gender, Ann Arbor staging, B symptoms, bulky disease and nodal involvement with treatment related mortality among patients who newly diagnosed with DLBCL treated with standard R-CHOP/CHOP as compared to R-CHOP/CHOP+MA/M/A regimen.

5) To identify the prognostic factors contributing to the effectiveness of treatment for patients who newly diagnosed with DLBCL.

CHAPTER 2

LITERATURE REVIEW

2.1 Diffuse large B-cell lymphoma

2.1.1 Definition and prevalence

Among all the lymphoid malignancy diagnosed in adults, diffuse large B-cell lymphoma or known as 'DLBCL' is the most common diagnosis. It is estimated that the incidence of Non-Hodgkin's Lymphoma (NHL) has reached be 15-20 cases/100,000 per year in US (Seer, 2015). Besides, data from WHO Classification of Tumour of Haematopoietic and Lymphoid Tissues fourth edition (Swerdlow et al., 2008) and International NHL study group (Armitage, 1997) indicated that DLBCL stands about one-third of all NHL in western countries and B-cell tumours reported globally. This figure maybe increasing as most of the DLBCL cases falls between sixty and seventy of age. The other types of aggressive lymphoma usually occur at lower median age, as for instance primary mediastinal lymphoma and Burkitt lymphoma . DLBCL contains highly proliferating large cells with basophilic cytoplasm, vesicular nuclei and prominent nucleoli. Based on 2008 WHO Classification, DLBCLs subdivided into molecular and immunophenotypic subgroups, morphological variants, as well as distinct disease entities (Swerdlow et al., 2008).

Currently, there are some cases of DLBCL which still remain undefined. Due to biologically heterogenous, there are no clear and accepted criteria for further

classification. Cases such as these are generally named as DLBCL, not otherwise specified (NOS). Using the principles established in 1994 by the Revised European American Lymphoma Classification (REAL), the current organization of DLBCL was designed (Harris et al., 1994) and adopted in the third and fourth editions of WHO Classification. It was thereafter that each type of lymphoma is categorized by the criteria of morphology, clinical data, cytogenetics, phenotype, molecular characteristics and by the identification of a normal counterpart.

2.1.2 Aetiology and risk factors

Currently, most causes of DLBCL were remain unknown. Nevertheless, cases arising de novo (referred as primary) should be clearly separated from those cases manifesting progression or transformation (referred to as secondary) of a less aggressive lymphoma. These included marginal zone lymphoma or nodular lymphocyte predominant Hodgkin lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma and follicular lymphoma. Congenital and acquired immunodeficiency states as well as agents causing molecular abnormality are predisposing factors for DLBCL (Hartge P, 2004).

A plethora of chemical substances, which involved fertilizers and pesticides (Weisenburger, 1985), and medical drugs also have been proposed to be causative agents. Alkylating agents are one of the culprit, whereby their uses are to treat solid tumours and haematological cancers. Incidence of lymphomas as secondary tumours

significantly increases with the concurrent use of alkylating agents and ionizing radiation, usually involving irradiation of large volumes of spleen or bone marrow.

DLBCL is a hereditary disease. It can be inherited in patients with immunologic deficiency diseases, and in families of patients with immunologic disorders (Vianna, 1977, Swerdlow et al., 2008). The incidence rate of diffuse aggressive lymphomas that affecting the brain seem to be higher and it has been reported in chronic patient who received immunosuppressant drugs, particularly after organ transplants. Indeed, patients who received alemtuzumab treatment for previous, unrelated haematological malignancies were also detected with EBV-positive DLBCL and it has been reported in a few trials (Abruzzo et al., 2002, Roch et al., 2008, Sohani et al., 2010). In term of characteristic, secondary DLBCL has similar features to that of early and polymorphic cases of post-transplant lymphoproliferative disorder (PTLD), whereby the sustainability of the remission may depend on the reduction in immunosuppressive therapy alone. Nevertheless, this is different from the therapeutic immunosuppression after solid organ transplantation. Prolongation of T-cell suppression by alemtuzumab may not result in rapid restoration of host anti-EBV and antitumour immunity once terminating the alemtuzumab therapy (Sohani et al., 2010). There is a difficulty in differentiating the influence of inheritance per se from immunosuppression in DLBCLs secondary to chronic iatrogenic immunosuppression. This may be of aetiological importance where there is no inherited background of the disease in the patient. Generally, Epstein-Barr virus (EBV) positivity usually manifest

in DLBCL emerging in the setting of one of these congenital or acquired immunologic disorders.

Notably, the association of DLBCL and viruses have become debatable over the last ten years. In fact, study has shown that there is a relationship between aggressive lymphomas and hepatitis-C virus (HCV) (De Vita et al., 1997) and a pathogenetic link has been suggested by another researcher (Luppi et al., 1998). Infection of HCV maybe linked to the malignant proliferation of defined B-cell subsets. The outcome survival of B-Non Hodgkin Lymphoma patients does not affected by HCV-related liver disease (De Vita et al., 1997).

Few types of lymphomas been identified to have linkage with human immunodeficiency virus (HIV)-infection (Cesarman, 2013). They are usually very aggressive and disseminated tumours occurring in an early phase of AIDS evolution. However, there is one malignancy that emerges in a latter phase of AIDS evolution; namely the primary central nervous system (PCNS) lymphoma with poor survival outcome. From the pathological aspects, lymphomas are divided into three categories. The first category involves lymphomas occurring more specifically in HIV positive patients (plasmablastic lymphoma (PBL) and pleural effusion lymphoma (PEL)]. The second group involves lymphomas occurring in other immunodeficiency states (PTLD-like polymorphic B-cell lymphoma). The last group was lymphoid tumours occurring in immunocompetent patients (usually Burkitt lymphoma and DLBCL) (Rossi et al., 2010). The number of lymphomas' cases among HIV positive patients have reduced

significantly while chemotherapy tolerability by the patients have increased with the initiation of the HAART therapy.

DLBCL which categorized under broad spectrum of lymphoproliferative disease was often associated with EBV. In regards of the two-provisional existence "ÉBV+DLBCL of elderly", some of the common conditions should be taken into treatment consideration, for example, primary effusion lymphoma, DLBCL associated with chronic inflammation, monomorphic PTLD, PBL, and lymphomatoid granulomatosis. Monomorphic PTLD is reported in patients starting at fifth decade of life without any underlying immunosuppression or previously diagnosed lymphoma. Oyama and colleagues described it as senescence-related immune system and the prognosis is not as good as the age-matched EBV-DLBCL (Oyama et al., 2007).

Lymphoid proliferations often related with human herpes virus 8 (HHV8). On top of PEL being the prototype, Castleman disease (CD) also shown to have association with HHV8 (Du et al., 2001). A peculiar form of DLBCL can emerge in the context of the multicentric variant of the latter, which frequently related to HIV infection. Monoclonal proliferation of HHV8+ lymphoid cells, which resembling plasmablasts and expressing IgM is able to sustain the form of disease. Even though the nomenclature plasmablastic is used, the cancer cells correspond to naïve, IgM-producing plasma cells without Ig somatic hypermutation. Conversely, PBL occurring in the oral buccal or other extranodal sites, usually indicate class-switched and

hypermutated Ig genes. This type of lymphoma must be differentiated from the earlier form as treatment may varies (Martelli et al., 2013).

2.1.3 Classification

DLBCL is a heterogeneous group of cancers. It contains cells with huge nuclei; usually bigger than those of tissue macrophages and at least double the size of a small lymphocyte. Involvement of extranodal sites may happen as well. Beside arising de novo, DLBCL may also emerge from the progression or transformation of a less aggressive B-cell neoplasm, such as follicular lymphoma, chronic lymphocytic leukemia, lymphoplasmacytic lymphoma, lymphocyte predominant Hodgkin lymphoma and marginal zone lymphoma. Four categories have been subdivided, some of which were not mentioned in previous schemes in the fourth edition of the WHO Classification of Haematopoietic and Lymphoid Tumours (Swerdlow et al., 2008); The categories including DLBCL- not otherwise specified (DLBCL, NOS), large cell lymphoma of terminally differentiated B-cells, DLBCL with predominant extranodal location and borderline cases. The organization of these neoplasms is quite complex as each category may further categorize according to morphology and/or clinic-pathologic aspects (Table 1).

Table 2.1: Classification of Diffuse Large B-cell lymphoma (DLBCL)

Diffuse large B-cell lymphoma (DLBCL), NOS • T-cell/histiocyte rich large B-cell lymphoma (T/HRBCL) • EBV+ DLBCL of the elderly
DLBCL with predominant extranodal location • Primary mediastinal (thymic) large B cell lymphoma (PMBL) • Intravascular large B-cell lymphoma (IVLBCL) • Primary cutaneous DLBCL, leg type (PCLBCL, leg type) • Primary DLBCL of CNS • Lymphomatoid granulomatosis
Large-cell lymphomas of terminally differentiated B-cells • ALK positive large B-cell lymphoma • Plasmablastic lymphoma (PBL) • Primary effusion lymphoma (PEL) • DLBCL associated with chronic inflammation
B-cell neoplasms with features intermediated between DLBCL and other lymphoid tumours • B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma • B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma

Note. Adapted from (Martelli et al, 2013).

2.1.4 Staging

Reviewing the tissue biopsy is crucial in making a diagnosis of DLBCL. For DLBCL diagnosis, excisional biopsy is a must; whereas core needle biopsy ought to be carried out only when other surgical approaches permitted in the case. In a suspected lymphoma presentation, the tissue sample can be obtained from enlarged superficial lymph node or on lymphoid tissue in Waldeyer's ring or on mediastinal adenopathy by core needle biopsy, mediastinoscopy or mediastinotomy (Andersson et al., 1992) or on retroperitoneal adenopathy by ultrasound-guided core needle biopsy, laparoscopy or

laparotomy (Pappa et al., 1996). After establishment of the diagnosis, the primary step is the evaluation before giving treatment as well as staging.

Staging system has been practiced since decades ago. Primarily, DLBCL staging used the standard system that was proposed at the Ann Arbor Conference in year 1971 (Carbone et al., 1971) and the Cotswlds modification (Lister et al., 1989). This staging system comprises the manifestation of B symptoms (fever > 38°C for at least three consecutive days, night sweats, unexplained body weight loss of more than 10% during the 6 months prior to diagnosis), the presence of extranodal disease, and amount of involvement sites and their relation to the diaphragm. The manifestation of B symptoms should be noted, and other symptoms may show specific sites of involvement. Besides, patients also need to be screened for their history and physical examination. A thorough physical examination is needed, which include assessment of all lymph node enlargement, recording site and size of all abnormal lymph nodes, evaluation of the presence of hepatosplenomegaly, inspection of the skin, detection of palpable masses, and inspection of Waldeyer's ring. Assessing patient's status with ECOG performance scoring is vital in all patients. This is particularly essential for the patients who involving in clinical trials.

The confirmatory of NHL disease required laboratory studies. NHL patients should be assessed for bone marrow reserves using a full blood count and a differential count of white blood cell. This is important as vigilant examination of the peripheral blood able to identify the presence of circulating lymphoma cells. Beside assessing the

liver and kidney function, lactate dehydrogenase (LDH) also play a pivotal role as parameter of tumour activity and it has been incooperated into the International Prognostic Index (Project, 1993). In fact, risk for urate nephropathy may be predicted by the urid acid level and all patients should have been assessed for their HIV, HBV, and HCV status as well.

Bone marrow aspirate and biopsy are compulsory assessments in confirming diagnosis. In order to improve 10-20% sensitivity of detection of NHL involvement, biopsy of the bilateral bone marrow have been suggested. Otherwise, a unilateral bone marrow specimen with the size of more than two centimeters is often enough. Additionally, MRI of the brain in patients with high risk of central nervous system (CNS) progression and lumbar puncture examination to assess liquor cytology identifying subclinical meningeal involvement maybe needed in diagnosing DLBCL.

The current standard practice for both response evaluation and staging is using ^{18}Fluorodeoxyglucose Positron Emission Tomography (PET). Plethora of studies showed that OS and PFS in aggressive lymphomas with or without residual masses detected with CT scan are highly predictive by the PET scan after completed the treatment. Besides, PET scan able to differentiate between lymphoma and necrosis or fibrosis in residual masses. Evaluation of combination Workshop Criteria (IWC) and PET was performed in a retrospective analysis of 54 patients with NHL. In the context of this study, new recommendations has been provided by the International Harmonization Project for response criteria for aggressive malignant lymphomas,

incorporating PET into the definition of response at the end of treatment (Juweid et al., 2007, Seam et al., 2007). PET scan should not be used in follow-up setting as it increases the rate of false positives and the fact that its increased sensitivity does not bring clinical benefit. Further evaluations are needed to assess the benefit of using PET and the cost effectiveness during the follow-up phase.

Additionally, "specific" staging studies that differ somewhat by site are needed especially for DLBCL developed in certain extranodal sites: this is particularly important in primary CNS lymphoma (Ferreri, 2013), gastric DLBCL (Ferreri and Montalban, 2007) and testicular DLBCL (Zucca et al., 2003).

Table 2.2: Ann Arbor staging

Stage	
I	Involvement of a single lymphatic region (I) or localized involvement of single extralymphatic organ or site (IE)
II	Involvement of two or more lymphatic regions on the same side of the diaphragm (II) or localized involvement of a single extralymphatic organ or site and of one or more lymphatic regions on the same side of the diaphragm (IIE)
III	Involvement of lymphatic regions on both sides of the diaphragm
IV	Diffuse or disseminated involvement of one or more extralymphatic organs with or without lymphatic involvement

Note. Adapted from (Tilly et al., 2015).

2.2 Prognosis

2.2.1 Clinical presentation and natural history

A variety of clinical presentation, treatment sensitivity and behaviour can be seen with each DLBCL category and their morphology and/or clinic-pathology variants aspects. Apparently, the presentation and natural history for each DLBCL category is difficult to report (Martelli et al., 2013). For DLBCL-NOS, it can be seen in adult patients, with a median age of 70 years old as well as in children (Swerdlow et al., 2008). Extranodal site plays an important part in determining the variable of clinical presentation, prognosis and behaviour of DLBCL-NOS (Moller et al., 2004). In general, patients rapidly manifest enlarging and usually have no symptomatic mass either at a single nodal or extranodal site. With regard to an extranodal organ, this presentation may account up to 40% of the patients (Moller et al., 2004). About 20% of patients who have these malignancies occur in localized manner and rarely disseminated, while 33% of patients manifested systemic symptoms. Overall, although DLBCL is an aggressive malignancy, yet it is potentially curable (Armitage, 1997). Patients with limited disease have a high cure rate of a 5-year progression free survival (PFS) ranging from 80% to 85%. On the other hand, patients with stage III/IV disease or symptomatic disease have a 5-year PFS of approximately 50% (Martelli et al., 2013).

2.2.2 Prognostic Factors

Prognostication of DLBCL can be categorized into those related primarily to the disease itself (eg. stage, tumour burden, proliferating fraction, extranodal involvement), the patient (eg. age and performance status), those related to aggressiveness indicators (eg. LDH serum level, β2-microglobulin levels, proliferating fraction), and those related to the treatment option. Nevertheless, involvement of extranodal sites may require special treatment options. These include the brain (Ferreri, 2011) or testis (Zucca et al., 2003), which constitute certain entities associated with poor prognosis. Besides, patient's outcome is highly predictive by response rate after initiation of primary treatment.

Based on clinical factors from more than 4000 patients, the International Prognostic Index (IPI) and age-adjusted International Prognostic Index (aaIPI) have been created as models for predicting outcomes of the disease (Project, 1993). In term of accuracy in predicting survival, IPI or aaIPI models have proven to be superior than the Ann Arbor classification. The aaIPI scoring which includes indicators such as LDH, performance status and stage, is the most commonly used in clinical practice and it is helpful in stratifying patients regardless of age. In fact, after the rituximab era, a revised version has been created (R-IPI) (Sehn et al., 2007). Nonetheless, in rituximab era, the IPI still remain the only benchmark of DLBCL prognosis although it has been documented to be robust in different series of DLBCL (Ziepert et al., 2010).

A plethora of prognostic factors for DLBCL have been identified; mainly biological parameters that is tumour related and indirectly to the host. This creates room for improvement in term of designing new therapeutic options as well as knowing more about the disease. Nevertheless, treatment decisions using these factors still remain investigational. Interestingly, biological heterogeneity in DLBCL can be obtained from the GEP of the malignant cells and their surroundings. In fact, some studies identified certain distinct subsets, including activated B-cell-like (ABC) and germinal-centre-like (GC) DLBCL to have different activation pathways, genetic markers, and clinical outcomes (Alizadeh et al., 2000, Shipp et al., 2002, Lenz et al., 2008). The BCL-6, MUM1 immunohistochemistry and CD10, have the same predictive abilities that enable them to be potential assistance for the gene expression profiles (Hans et al., 2004). At present, more data is needed for the practice of GEP or immunohistochemistry in the clinical settings. Nevertheless, some preliminary data from Dunleavy suggested that certain drugs are found to be more active in one of the GEP subgroups of recurrent DLBCL compared to the other (Dunleavy et al., 2009). A recent retrospective study also showed that ABC-DLBCL patients with respect to GCB-cell-DLBCL receiving lenalidomide as salvage therapy were significantly showed better response rate (Hernandez-Ilizaliturri et al., 2011). However, larger prospective type of studies are needed to confirm the prognostic value of these markers.

2.3 Chemotherapy

Chemotherapy has a pivotal role in cancer treatment whereby the standardized chemotherapeutic regimen uses anti-cancer drugs whether as monotherapy or combination therapy. The purpose of chemotherapy given is to cure the disease, or perhaps to reduce symptomatology (palliative chemotherapy) or to prolong life. Based on the cytotoxicity properties, chemotherapeutic agents able to interrupt cell division (mitosis) but the susceptibility of cancer cells to these agents are variable (Kehe et al., 2009, Malhotra and Perry, 2003). To some extent, chemotherapy can be treat as an option to pose stress to cells or terminate them, which may then lead to cell death or apoptosis (Makin and Hickman, 2000).

Normal cells are rapidly dividing and are thus sensitive to anti-mitotic drugs which may subsequently damage by the chemotherapy. These include those cells in the hair follicles, bone marrow and digestive tract. As a result, some of the commonly reported side-effects of chemotherapy including alopecia (hair loss), myelosuppression (decreased production of blood cells, hence also immunosuppression), and mucositis (inflammation of the lining of the digestive tract) (Rajesh K. Saini, 2012). Nevertheless, these side effects are temporary and do not prolong after chemotherapy. Chemotherapy drugs have some effect on immune cells (especially lymphocytes). This enable chemotherapy drugs to be used in a host of diseases that result from harmful over activity of the immune system against self or termed as autoimmunity. The autoimmune

diseases may include multiple sclerosis, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), vasculitis and many others (Zack, 2012).

2.3.1 Cyclophosphamide

Cyclophosphamide is used as a chemotherapeutic agent besides suppressing the immune system. In the field of hematology, it is used to treat lymphoma, leukemia multiple myeloma. Meanwhile in oncology, solid tumours such as breast cancer, sarcoma, ovarian cancer, and small cell lung cancer can be treated by cyclophosphamide. Besides, cyclophosphamide also has a role in nephrotic syndrome and following organ transplant due to its immunosuppressive effect. Cyclophosphamide can be taken intravenously or even enterally . Majority people experience some sort of side effects from the used of cyclophosphamide. Common side effects of cyclophosphamide include loss of appetite, low white blood cell counts, hair loss, vomiting and bleeding from the bladder. Severe side effects were also documented which include pulmonary fibrosis, fertility problems, allergic reactions and increased future risk of cancer. Cyclophosphamide originated from two groups of drugs, mainly alkylating agent and nitrogen mustard. The mechanism of action involves interfering with the replication of DNA and the development of RNA (McEvoy, 2004).

Absorption of oral cyclophosphamide is fast which then converted to active metabolites in the liver. This process is catalyzed by mixed-function oxidase enzymes (cytochrome P450 system) (Cohen and Jao, 1970, Huttunen et al., 2011) where the main