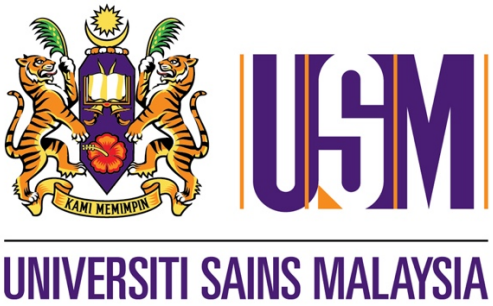

The role of ^{18}F -FDG PET-CT in assessing bone marrow involvement in Diffuse Large B-Cell Lymphoma (DLBCL)

by

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Dissertation Submitted in Partial Fulfilment
Of the Requirements for The Degree Of
Master of Medicine (Nuclear Medicine)

ADVANCED MEDICAL AND DENTAL
INSTITUTE UNIVERSITI SAINS MALAYSIA

2018

DECLERATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Nuclear Medicine. It is also not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.

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Dr Siti Maisarah Mohd Nasir

P-IPM0008/14

ACKNOWLEDGMENT

I would like to start by taking this opportunity to express my greatest gratitude and acknowledgement to the Kementerian Kesihatan Malaysia and Advanced Medical and Dental Institute, Universiti Sains Malaysia for giving me the opportunity to be a part of this 4 years master programme that has eventually lead me to the completion of this dissertation.

My greatest appreciation and respect to my mentors and lecturers in Universiti Sains Malaysia and Hospital Pulau Pinang who have dedicated their time, knowledge and skills towards me throughout this 4 years. Specifically to Dr Mahayuddin Abdul Manap and Dr Fadzilah Hamzah for being my supervisors thus helping me in the completion of this dissertation. My most appreciation to Dr Mohd Wajdi Bin Ghazali for his time and effort in being one of the readers. I would also like to convey my gratitude to Dato' Dr Goh Ai Sim and Dr Gan Ee Leng from Haematology Dapartment, Hospital Pulau Pinang, Dr Hakimah Bt Mahsin @ Ahmad Nasir and Dr Ida Marhainis Bt Isahak from Pathology Department Hospital Pulau Pinang for their kind assistance in helping me to complete this dissertation as well as Encik Nizuwan Bin Azman from Advanced Medical and Dental Institute, Universiti Sains Malaysia for his valuable advice on statistical analysis. Of course not forgetting all my fellow colleagues, specialists, nurses, technologists, pharmacists, physicists and the rest of the crew whom has been extremely wonderful throughout my journey in Hospital Pulau Pinang.

A special mention is needed especially to my loving husband Dr Fahmi Pramaditto Bin Azmi, my two wonderful children and beautiful mother Nor Hasmah Bt Nor Hashim for always supporting me, standing by me and encouraging me to complete this 4 years. Also to my fellow batch mates Dr Lee Yeong Fong, Dr Nor Qistin Muhamad, Dr Lee Kwang Wei and Dr Abdullah Hanif Rosdi with whom I have started this journey with and wished we all had been able to complete this journey together. Last but not least, to all my family members and friends who have advised me and have been with me through all the difficult times and especially to those who had to bear my absence at times due to my responsibilities.

Thank you all again and may Allah S.W.T always bless us.

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ABREVIATIONS

^{18}F	Fluorine-18
BCCA	British Colombia Cancer Agency
BMB	Bone Marrow Biopsy
BMI	Bone marrow involvement
CI	Confidence interval
CNS	Central Nervous System
CT	Computed Tomography
DLBCL	Diffuse Large B-cell Lymphoma
ESMO	European Society of Medical Oncology
FDG	Fluorodeoxyglucose
6P	6 - Phosphate
FN	False Negative
FP	False Positive
GE	General Electric
GLUT	Glucose Transporter

HPP	**Hospital Pulau Pinang**
IPI	**International Prognostic Index**
MBq	**Megabecquerel (SI)**
mCi	**Millicurie (SI)**
MIP	**Maximum Intensity Projection**
MRI	**Magnetic Resonance Imaging**
NCCN	**National Comprehensive Cancer Network – IPI**
NPV	**Negative predictive value**
PET	**Positron Emission Tomography**
PPV	**Positive predictive value**
ST	**See and Treat**
SUV_{max}	**Standard Uptake Value Maximum**
TN	**True Negative**
TP	**True Positive**

ABSTRAK

Pendahuluan: Dalam Limfoma Sel-B Besar Difus (DLBCL) yang baru didiagnosis, PET-CT ^{18}F-FDG memang digunakan secara rutin di seluruh dunia bagi tujuan pengkelasan perubatan. Penglibatan sumsum tulang adalah penting dalam pengurusan dan keperluan terapeutik. Standard emas dalam pengambilan sumsum tulang dikenali sebagai biopsi sumsum tulang. Kajian kohort ini berperanan menilai PET-CT ^{18}F-FDG dalam pengesanan penglibatan sumsum tulang secara relatif berbanding kepada biopsi sumsum tulang.

Objektif: Kajian ini dijalankan bagi tujuan untuk membuktikan penggunaan imbasan PET-CT ^{18}F-FDG berguna dalam menilai penglibatan sumsum tulang dalam kes DLBCL selaras dengan biopsi sumsum tulang.

Kaedah: Semua pesakit yang dirujuk dari rantau utara Malaysia yang baru didiagnos dengan DLBCL bagi tujuan pengkelasan tulang sumsum pra-kemoterapi. Semua pesakit yang dirujuk akan menjalani imbasan PET-CT ^{18}F-FDG dan biopsi sumsum tulang sebelum rawatan kemoterapi diberikan. Kedua-dua ujian mesti dilakukan dalam jangkamasa yang tidak melebihi 60 hari.

Keputusan: Daripada 28 pesakit yang dirujuk untuk kajian ini, 21 pesakit berjaya didaftarkan. Sejumlah 14 pesakit menunjukkan imbasan PET-CT ^{18}F-FDG negatif dan biopsi negatif, 3 pesakit menunjukkan imbasan

PET-CT ^{18}F-FDG positif dan biopsi positif dan 4 pesakit lain menunjukkan imbasan PET-CT ^{18}F-FDG positif tetapi biopsi sumsum tulang negatif. Imbasan PET-CT ^{18}F-FDG menunjukkan nilai ramalan negatif 100% dan sensitiviti 100%. Terdapat persetujuan yang sederhana antara skan PET-CT ^{18}F-FDG dan BMB yang menunjukkan kesesuaian penting (kappa k-value: 0.500, $p < 0.008$).

Kesimpulan: Imbasan PET-CT ^{18}F-FDG dalam pola pengambilan selektif (negatif / multifokal / fokus) dapat memainkan peranan sebagai alternatif bagi teknik invasif seperti biopsi sumsum tulang dalam penglibatan pengkelasan sumsum tulang dalam kes DLBCL.

ABSTRACT

Title : The role of ^{18}F -FDG PET-CT in assessing bone marrow involvement in Diffuse Large B-Cell Lymphoma (DLBCL).

Introduction: In newly diagnosed DLBCL, ^{18}F-FDG PET-CT is routinely used worldwide for staging. Bone marrow involvement is crucial in order for therapeutic purposes. Bone marrow biopsy is known as the gold standard in ruling out bone marrow involvement. This cohort study hereby assesses the role of ^{18}F-FDG PET-CT in the detection of bone marrow involvement comparatively to bone marrow biopsy.

Objective: The aim of this study is to justify the usage of ^{18}F-FDG PET-CT scan in assessing bone marrow involvement in DLBCL as appose to bone marrow biopsy.

Methods: All newly diagnosed DLBCL cases within the northern region of Malaysia was referred for pre-chemotherapy bone marrow staging. All subjects underwent both ^{18}F-FDG PET-CT scan and bone marrow biopsy before chemotherapy treatment was given. Both studies were done between an interval of not more than 60 days.

Results: Out of 28 subjects referred for this study, 21 subjects were successfully enrolled. A total of 14 subjects showed a negative ^{18}F-FDG PET-CT scan and BMB, 3 subjects showed a positive ^{18}F-FDG PET-CT scan and BMB and another 4 subjects showed a positive ^{18}F-FDG PET-CT scan but negative BMB. ^{18}F-FDG PET-CT scan showed a negative

predictive value of 100% and sensitivity of 100%. There was moderate agreement between ^{18}F-FDG PET-CT scan and BMB which showed significant concordance (kappa k-value: 0.500, $p<0.008$).

Conclusion: ^{18}F-FDG PET-CT scan in selective pattern of uptake (negative / multifocal / focal) is an alternative method comparatively to BMB for assessing baseline bone marrow involvement among DLBCL patients.

1.0 Introduction & Literature review

Lymphomas which is part of the neoplasm group comprising of Hodgkin's Lymphoma (HL) and Non-Hodgkin's Lymphoma (NHL) in which the management and prognostic value is dependent on the stage, severity and extension of the lymphoma (Pelosi *et al.*, 2008). Diffuse large B-cell lymphoma (DLBCL) is the most common form of lymphoma and it attributes up to 58% with an overall annual incidence of 25000 cases (Berthet *et al.*, 2013). Statistically DLBCL contributes to 5% of all type of malignancies (Adams *et al.*, 2014a). Two studies in Malaysia written by Peh *et al.* (2003);Chang *et al.* (2006) agreed that DLBCL had a higher prevalence compared to other types NHL. Statistically, in Horesh and Horowitz (2014) they revealed a male predominance in NHL cases within the United States, United Kingdom and Europe which is similar to the findings of an article written by Nair *et al.* (2016) where there is a higher male to female ratio globally inclusive of Asian countries. In Fadilah (2009) she also acknowledges a higher prevalence in male gender patients compared to women for NHL in Malaysia. She also highlighted NHL being the 3rd commonest cancer in males less than 50 years of age followed by the 10th commonest in males more than 50 years of age. Quite different for the female gender which is lesser and contributes to being the 10th commonest cancer in the age group less than 50 years old and 14th place vice versa. This suggests that NHL is more aggressive in males compared to females.

DLBCL is a highly aggressive disease that needs to be detected and treated immediately. The disease's clinical course is highly variable

based on the various subtypes and in most cases presented late at Stage III or IV (Alizadeh *et al.*, 2000) with up to 40% of patients relapsing after the 1st line chemotherapy (Johnson *et al.*, 2015).

Multiple risk factors contributing to the incidence of DLBCL includes autoimmune disease, immunosuppressive disease such as human immune-deficiency virus infection, hepatitis C virus seropositivity, history of lymphoma in family, occupational exposure, and increased body mass as a young adult (Tilly *et al.*, 2015). According to its pathogenesis it arises either from the B-cell germinal centre or from a later differentiation of an aggressive mature B lymphocytes malignancy (Alizadeh *et al.*, 2000).

Since early 2014, most physicians often refers to the European Society of Medical Oncology (Tilly *et al., 2015*) clinical practice guideline as the standard reference in diagnosing DLBCL (Tilly *et al.*, 2015). Physical examination, performance status (Zhou *et al., 2014*), assessment of B symptoms, haematological laboratory references including immuno-phenotypic investigations as well as protein electrophoresis, and imaging work groups has played a major part in the making of treatment decisions. The initial staging was initially made based on the Ann Arbor classification which was initially implemented in the 1970's followed by further alteration in the 1980's known as Modified Ann Arbor (Cotsworlds) as seen in Table 1.1 (Johnson *et al.*, 2015). The International Prognostic Index (IPI) group was introduced to determine prognostic outcomes of patients (Berthet *et al.*, 2013) as seen in Table 1.2. In the IPI index, lymphoma was classified into scorings per 5

characteristics where one of them includes bone marrow involvement which is categorized as extra-nodal sites and is used to risk stratify and identify risk discrete categories (Khan *et al.*, 2013; Zhou *et al.*, 2014). Treatment then given to NHL patient's was decided based on age and IPI index which was classified into young low-risk without bulky disease (IPI=0), young low-risk with bulky disease (IPI=0), young low-intermediate risk (IPI=1), young high and high-intermediate risk (IPI=2), patient aged 60-80 years old, patient >80 years old and CNS involvement prophylaxis (Tilly *et al.*, 2015). In 2014, a revision of Ann Arbor staging (Lugano 2014) as shown in Table 1.3, was done in order to achieve more congruous therapeutic response (Van Heertum *et al.*, 2017).

Stage	Description
I	Single lymph node group
II	Multiple lymph node groups on same side of diaphragm
III	Multiple lymph node groups on both sides of diaphragm
IV	Multiple extranodal sites or lymph nodes and extranodal sites
X	Bulk >10cm
E	Extranodal extension or single isolated site of extranodal disease
A/B	B symptoms: weight loss >10%, fever, drenching night sweats

Table 1.1: Ann Arbor Classification (Cotsworlds).

Risk Factors (Serum LDH > normal, Stage III – IV, Performance status 2-4)	Risk Categories	Estimated 3 year overall survival (95% CI)
0	Low	98%
1	Low intermediate	92%
2	High intermediate	75%
3	High	75%

Risk Factors (Age > 60 years, Serum LDH > normal, Stage III – IV, Performance status 2-4, Extranodal site > 1)	Risk Categories	Estimated 3 year overall survival (95% CI)
0-1	Low	91%
2	Low intermediate	81%
3	High intermediate	65%
4-5	High	59%

*Age-adjusted IPI in patients less than 60 years old

Table 1.2: International Prognostic Index (IPI)

Stage	Involvement
Limited	
I	One node @ group of adjacent nodes **Extranodal (E) status = single extranodal lesion without nodal involvement*
II	2 @ more nodal groups on the same side of the diaphragm **Stage I and II by nodal extent with limited contiguous extranodal involvement*
II bulky *	as above with “bulky” disease
Advanced	
III	Nodes on both sides of the diaphragm; nodes above the diaphragm with spleen involvement
IV	Additional non-contigous

Table 1.3 Modified Ann Arbor (Lugano 2014)

Bone marrow involvement affects more commonly in NHL in particular DLBCL subtypes with incidence up to 17% (Carr *et al.*, 1998). It contributes to 1 point in the IPI index score and is found in up to 15% of newly diagnosed DLBCL cases (Chen *et al.*, 2018). The involvement in the marrow is usually categorised into large cells for example concordant (prominent component of large transformed high grade lymphoma) BMI or small cells for example in discordant (small lymphoid cells with cleaved nuclear contours suggestive of coexisting low grade lymphoma) BMI (Chung *et al.*, 2007; Adams and Kwee, 2015). In another study by Adams *et al.* (2015b) lymphomatous cells which infiltrate the bone marrow results from a haematogenous spread or adjacent soft tissue extension and is more aggressive which changes the prognostic and therapeutics consequences. In a study by Sehn *et al.* (2011) their findings were in tandem with other previous studies that concordant bone marrow involvement has poor prognostic value for overall survival. The study also suggested a significant prolonged PFS (patient free survival) value by adding rituximab when an early bone marrow involvement is detected (Sehn *et al.*, 2011). Therefore, the accuracy in BMI staging is paramount in order for therapeutic strategy optimization (Berthet *et al.*, 2013).

For diagnosing lymphoma, surgical excisional biopsy remains to be the optimal choice as it allows nodal assessment and dispenses adequate sample for phenotypic and molecular studies (Tilly *et al.*, 2015). In assessing BMI, BMB or also known as marrow trephine biopsy plays a pivotal role in routine staging (Carr *et al.*, 1998). Furthermore,

this diagnostic intervention was known to be the gold standard in determining bone marrow status (Berthet *et al.*, 2013). In Adams *et al.* (2015a), BMB is a definitive proof and known to aid in diagnosing DLBCL that has no appropriate extramedullary lesion for biopsy. The detection of BMI through BMB histologically has acclaimed an unfavourable prognostic factor. Thus, by obtaining histologically proven BMI results via BMB, it indicates an advanced disease assisting to a selection of a more intense therapeutic regime. Its ability to occasionally diagnosed other bone marrow disease or alterations is also an advantage (Adams *et al.*, 2015c). However, it is a blinded painful invasive procedure which can lead to risks of pain, anxiety, haemorrhage and infection (Abdelrahim *et al.*, 2018). Inadequate sampling due to factors such as wrong biopsy site and technique also plays a major part of downside in bone marrow trephine biopsy (Abdelrahim *et al.*, 2018). In previous studies, particularly in high grade NHL, unilateral iliac crest trephine biopsy consistently shows unreliability and discrepancy reaching up to 50% (Carr *et al.*, 1998). In Adams *et al.* (2015a), the study showed a low positive marrow histology incidence of only 17%. Apart from the limitation of high numbers of sampling errors, the procedure is also known to be time consuming (Adams *et al.*, 2015c).

In the latest consensus established by international conference of malignant lymphoma (Lugano classification) working groups, the role of Fluorine-18 Fluorodeoxyglucose positron emission tomography – computed tomography (^{18}F-FDG PET-CT) scan is the current gold standard of staging and restaging in lymphoma (Tilly *et al.*, 2015). This

allows physiological response assessment based on the tumour metabolism itself (Van Heertum *et al.*, 2017). ^{18}F (Fluorine-18) is a positron emitting isotopes used commonly in oncology for primary staging and tumour treatment response. (Adams *et al.*, 2014a). It is a glucose analogue whereby the uptake is increased in the tumour cells in relation to raised glucose transfer (Carr *et al.*, 1998). ^{18}F enters the cells through sodium-independent glucose transporter (GLUT-1, GLUT-3 and GLUT-12) via facilitated diffusion. After entering the cells, it undergoes the normal glucose metabolic pathway where hexokinase phosphorylates and forms itself into 2- ^{18}F fluoro-2-deoxy-d-glucose-6-phosphate (^{18}F FDG-6P). At this point, the ^{18}F metabolic pathway defers as compared to the previous normal glucose pathway. Firstly, the fructose-6-phosphate is converted from glucose-6-phosphate by phosphoglucose isomerase, then further irreversibly phosphorylates to fructose-1,6-biphosphate by the phosphofructokinase and further continues in the glycolytic pathway. However, compared to normal glucose pathway that undergoes glycolysis, the presence of charged phosphate group causes the ^{18}F FDG-6P to be trapped and unable to leave the cell. Therefore, as tumour cells glycolysis rate increases, ^{18}F FDG-6P accumulates in tumours than normal tissue.

In PET, a study is evaluated based on the size and intensity where visual assessment by Standard Uptake Value (SUV) is used to determine any abnormal FDG accumulation seen in ^{18}F-FDG PET-CT images. This is the measurement uptake of the tumour based on a distribution volume. The volume of interest which is equivalent to the

voxel with highest uptake in a tumour or lesion is analysed using maximum SUV measure SUV_{max} in which must be identified on reconstructed initial ^{18}F-FDG PET-CT images (Boellaard *et al.*, 2010). CT on the other hand gives morphological and anatomical visualization for better accuracy of anatomical correlation, degree and lesion characterisation by ^{18}F-FDG PET-CT. Therefore, integrated PET-CT carries out morphological and functional properties in one single imaging (Boellaard *et al.*, 2010). For bone marrow involvement in lymphoma, there will be increased bone marrow ^{18}F-FDG PET-CT uptake exceeding liver FDG uptake (Adams *et al.*, 2014a).

In lymphoma, ^{18}F follows the red marrow and is further distributed throughout the skeletal system (Adams *et al.*, 2014a). Therefore ^{18}F-FDG PET-CT is able to provide high-quality whole-body imaging for primary staging, remission assessment and treatment of lymphoma (Carr *et al.*, 1998; Cheson *et al.*, 2007). ^{18}F-FDG PET-CT scan is a non-invasive technique, well known to be highly sensitive in lymphoma disease especially in DLBCL (Pregno *et al.*, 2012). One of the other advantages is that it promotes visualization of the whole bone marrow in comparison to restricted site of BMB (Adams and Kwee, 2015) hence being able to achieve simultaneous staging of extramedullary disease (Adams *et al.*, 2015c) . In assessing the BMI in ^{18}F-FDG PET-CT, the pattern of uptake plays a major role in determining diagnostic and prognostic value in DLBCL cases. In Chen *et al.* (2018) they reported the implication of BMI in patients prognosis depends on the type of uptake seen on ^{18}F-FDG PET-CT scan. Type of uptake pattern was

defined as focal (SUV_{max} is more than liver), diffuse (homogenously diffuse SUV_{max} more than liver) and normal (homogenously diffuse SUV_{max} less than liver). Their study demonstrated focal uptake of pattern as an independent predictor for inferior outcomes suggesting BMB is no longer necessary in the DLBCL management. Tilly *et al.* (2015) the focal pattern of uptake in ^{18}F-FDG PET-CT is highly specific with better sensitivity compared to BMB in DLBCL suggesting that BMB is no longer required in determining BMI. However, not all FDG avid bone marrow abnormality represents lymphoma as explained in Adams *et al.* (2015c) alongside other limitations of ^{18}F-FDG PET-CT such as lack of histologic material and the use of ionizing radiation.

Other modalities such as CT scans and magnetic resonance imaging (MRI) may also be utilised in detecting BMI in DLBCL cases. In most practices, CT scan is done in the initial staging of lymphoma disease. In Ahmed *et al.* (2013), he explained that the presence of skeletal lesions seen on CT are likely due to cortical bone metastasis. This is usually seen in the advanced stages of the disease with variable osseous appearances (lytic/sclerotic/mixed) seen on CT followed by further sclerosis development seen post chemo-radiotherapy (Johnson *et al.*, 2015). In an article by Adams *et al.* (2015d) CT shows lesser sensitivity in BMI detection and the presence of tumour-induced cortical bone destruction showed no prognostic implication in DLBCL as osteolysis is rare in most patients with BMI. Thus, CT scan is more anatomical and preferably used for better delineation of lymphadenopathy and its

measurements, detection of thrombosis/compression as well as radiation planning (Tilly *et al.*, 2015).

Another modality known as MRI can also be used for baseline staging of BMI in lymphoma. In MRI, it is able to provide high spatial resolution images in detecting BMI which shows a T1-hypointense mass in MRI images as described in Adams et al, (2013);Johnson et al, (2015). Large studies like Adams *et al.* (2013), concluded that there is not much difference between MR imaging and ^{18}F-FDG PET-CT scan in staging for BMI. Like ^{18}F-FDG PET-CT scan it is equivalently sensitive with a favourable negative predictive value (NPV). However, MRI imaging showed lesser sensitivity in high grade lymphoma than in low grade lymphoma compared to ^{18}F-FDG PET-CT imaging (Adams *et al.*, 2013).

In most previous studies, evidence has suggested that BMB may be replace by ^{18}F-FDG PET-CT in Hodgkin's lymphoma. The low incidence rate of BMI and lack of treatment consequences in advanced HL disease makes it more permissible for BMB to be omitted in assessing BMI provided ^{18}F-FDG PET-CT scan is done (Adams *et al.*, 2015c). The value is obsolete and has already been practiced in some centres. (El-Galaly *et al.*, 2014). In Cheson *et al.* (2007), the study recommends ^{18}F-FDG PET-CT scan to be carried out for pre-treatment patients in potentially curable lymphomas which is commonly FDG avid, such as DLBCL and HL in order of better delineation of the disease. In previous studies such as Berthet *et al.* (2013) involving a homogenous population of 133 patients which described BMI assessment done by ^{18}F-FDG PET-CT scan is superior in diagnostic capability and stratifying

patient's prognosis compared to BMB with a sensitivity of 93.9% vs 24.2% and similar specificity of 99% vs 100% in newly diagnosed DLBCL. Unlike Adams *et al.* (2014b), his study showed that both quantitative as well as qualitative ^{18}F-FDG PET-CT scan in assessing BMI are inferior to BMB in prognosis stratification with a sensitivity of 68.1%. These conflicting results as well as limitations to cost, availability and limited study produced during their time were the main reasons as to why BMB was still mandated for assessing BMI (El-Galaly *et al.*, 2014). However, in further recent studies by Adams *et al.* (2014a) based on a systemic and meta-analysis review he reported that evidence suggest ^{18}F-FDG PET-CT scan is a substitute for BMB in diagnosing BMI in DLBCL. Nevertheless, in Chen-Liang *et al.* (2015), they too proposed that a BMB is no longer required in a positive focal pattern ^{18}F-FDG PET-CT scan (classified stage IV). Another article by El Karak et al., (2017), he suggested to omit the need for BMB in a negative for BMI ^{18}F-FDG PET-CT scan in view of the high NPV. In a recent study by Chen et al., (2018), in view of BMB's unfavourable accuracy and limitation of predicting patient prognosis compared to ^{18}F-FDG PET-CT scan, the role of BMB is unnecessary in assessing BMI.

Despite multiple studies done in assessing BMI with ^{18}F-FDG PET-CT scan, the role of pre-chemotherapy ^{18}F-FDG PET-CT in assessing BMI in Malaysia has yet to be enforced in view of limited sources i.e. limited availability of PET-CT scans, affordability and awareness by primary physician teams. All lymphoma patients in Malaysia is still subjected to BMB despite current literature suggests and allows

practitioners to be more selective in deciding which patient is more suitable for BMB if the role of ^{18}F-FDG PET-CT is taken into consideration. The motive of this study is to look into the ability of ^{18}F-FDG PET-CT as well as to collate the sensitivity and specificity between ^{18}F-FDG PET-CT scan and BMB in assessing BMI specifically in DLBCL.

2.0 RATIONALE/BENEFITS OF THE STUDY

This research is conducted to determine the role and significance of ^{18}F-FDG PET-CT scan in assessing BMI for DLBCL disease. With ^{18}F-FDG PET-CT imaging, it has the ability to represent as another replacement tool besides BMB for detecting the presence of BMI in DLBCL. Detection of BMI via ^{18}F-FDG PET-CT scan imaging, could possibly be more time saving and more convenient for the patient as it can minimize the need for an invasive procedure.

2.1 Aim

To justify the use of ^{18}F-FDG PET-CT scan in assessing bone marrow involvement in DLBCL as appose to bone marrow biopsy.

2 2. Objectives

2.2.1 General Objective:

. To validate the presence of BMI in DLBCL based on routine staging ^{18}F-FDG PET-CT scan, is as sufficiently accurate as compared to BMB.

2.2.2 Specific Objectives:

. To describe into the demographics of patients with DLBCL that are referred for ^{18}F-FDG PET-CT in Hospital Pulau Pinang.

. To compare the sensitivity & specificity between ^{18}F-FDG PET-CT and bone marrow biopsy in detection of BMI.

. To determine the characteristics of ^{18}F-FDG uptake that positively demonstrates BMI.

2.3 Hypothesis Statement

- Null hypothesis: The use of ^{18}F-FDG PET-CT does not serve as an alternative method comparatively to bone marrow biopsy for baseline bone marrow assessment in Diffuse Large B-cell lymphoma patients.

- Alternate hypothesis: The use of ^{18}F-FDG PET-CT does serve as an alternative method comparatively to bone marrow biopsy for baseline bone marrow assessment in Diffuse Large B-cell lymphoma patients.

3.0 METHODOLOGY

3.1 Study Design, Study location and Study Period

A prospective study with universal sampling method was carried out in the Nuclear Medicine Department, Hospital Pulau Pinang from the period of November 2016 to February 2018.

3.2 Study Sample

Subjects referred to Hospital Pulau Pinang who were recently diagnosed with DLBCL were the reference population. Source population were subjects who were referred to the Nuclear Medicine Department, Hospital Pulau Pinang for first diagnostic pre-chemotherapy ^{18}F-FDG PET-CT scan. The subjects with newly diagnosed DLBCL who came to Nuclear Medicine Department, Hospital Pulau Pinang for first diagnostic^{18}F-FDG PET-CT scan was the sampling population and the study participants were those whom have consented to participate in this study and fulfilled the inclusion and exclusion criteria.

3.3 Inclusion and Exclusion Criteria

3.3.1 Inclusion criteria:

. Adult (>18 years old) patients newly diagnosed with Diffuse Large B-cell lymphoma (DLBCL).

. Pre-treated Diffuse Large B-cell lymphoma (DLBCL) and underwent ^{18}F-FDG PET-CT as well as bone marrow biopsy.

3.3.2 Exclusion criteria:

. Subjects previously known & treated for lymphoma.

. Subjects which have passed interval between first FDG-PET/CT and BMB of more than 60 days.

. Subjects who have received hematopoietic growth factor injections less than 48 hours before the first ^{18}F-FDG PET/CT scan.

. Subjects with other primary malignancies.

3.4 Data Collection

All referrals for pre-staging ^{18}F-FDG PET-CT in newly diagnosed Diffuse Large B-cell Lymphoma (DLBCL) were screened by the principal investigator (PI). Base on the referral forms that was sent, those who fulfilled the inclusion and exclusion criteria were recruited to participate in this study and informed consents were taken. Any bone marrow biopsy that was done prior to the scan, was given ^{18}F-FDG PET-CT appointment within the period of 60 days following the biopsy. A delay period interval of more than 60 days may result in morphological histopathological changes leading to invalidity of results (Dupas et al., 2013). PET-CT scan appointment is scheduled 48 hours after any hematopoietic growth factor injections to avoid false positive bone marrow uptake in PET-CT scan secondary to inflammatory response (Hanaoka et al., 2011). Subjects preparation for PET-CT was run per department protocol. Subjects was advised to reduce carbohydrates in their meal and all patients fasted 4-6 hours before scan day to limit the impact and avoid glucose to compete with ^{18}F-FDG during scan. Instruction to avoid strenuous exercise for few days before study was also given to reduce muscle uptake will also be conveyed.

On the day of the appointment, subjects were clerked and examined to ensure compliance to the preparation and clinical data as well as written consent were obtained. Subjects with diabetes was carefully scheduled in the morning before taking insulin and those with metformin medication was advised to withheld medication from

morning of the scan to decrease bowel background activity related to the drug. Before injection of FDG, all subjects was ensured that their blood glucose levels were less than 11 mmol/L as indicated in the department protocol.

A 20'gauge size branula was then inserted and subjects was given radiopharmaceutical intravenous injection ^{18}F-FDG per the routine standard dose (6MBq/kg; 10-15mCi). Each subject was explained that ^{18}F-FDG causes no significant risks whereby ^{18}F-FDG has been widely used with high safety profile. However, subjects were explained regarding small possible hypersensitivity reactions such as itchiness, skin rashes, hypotension, chills, nausea, fever and vomiting. Bleeding, bruising, discomfort, infection and/or pain at needle site caused by the needle puncture for injection of radiopharmaceutical was also explained to the subject. Thus, immediate medical care and attention was ensured to be provided if such events occur.

The standard practice for evaluation and staging of DLBCL in HPP is by PET CT scan using a radiopharmaceutical agent of ^{18}F. Imaging is done 45-60 minutes post injection (longer delays increase tumour to background ratios) which involves entire base of brain till upper thigh. PET (GE discovery ST) scanning was done from mid femur to the vertex with 3 min per bed position. A transmission date acquisition of 40-minutes attenuation data acquisition using built in CT scanner is done along with 1-hour attenuation data acquisition using radioactive sources with 2D mode (in septa) collimator and

30% energy window of 511kev. The low-dose CT data was used for attenuation correction of the PET images. Scan results were seen by two experienced appointed specialists in the same centre and both of them was involved in assessing BMI in the scans based on their uptake. Any disagreement in the results was resolved by a third reader a senior consultant of Nuclear Medicine to attain an anonymous definite result. Results of the findings were then compared with traced BMB findings (acquired from Pathology Department HPP and Haematology Department HPP). Study was suspended or terminated once ^{18}F-FDG PET-CT scan and bone marrow biopsy by haematology team was completed.

3.5 Bone marrow involvement and uptake

The assessment of BMI is done by utilising 2 different methods:

1. Qualitative method
2. Quantitative method

3.5.1 Qualitative method:

- Interpretation by a specialist appointed - blinded to BMB and ^{18}F-FDG PET-CT scan results
- Any FDG uptake more than liver FDG uptake is interpreted as abnormal (= lesion).

Based on a the study by (Adams *et al.*, 2015a), bone marrow involvement is classified as:

- Uni-focal: 1 circumscribed lesion

- Multifocal: > 1 circumscribed lesion – Figure 3.1

- Diffuse: Whole bone marrow (rather than a single location) - Figure 3.2

- Focal & Diffuse: Circumscribed & Diffused area – Figure 3.3

- Negative : No lesions / Diffuse bone marrow uptake with SUV_{max} less than liver – Figure 3.4

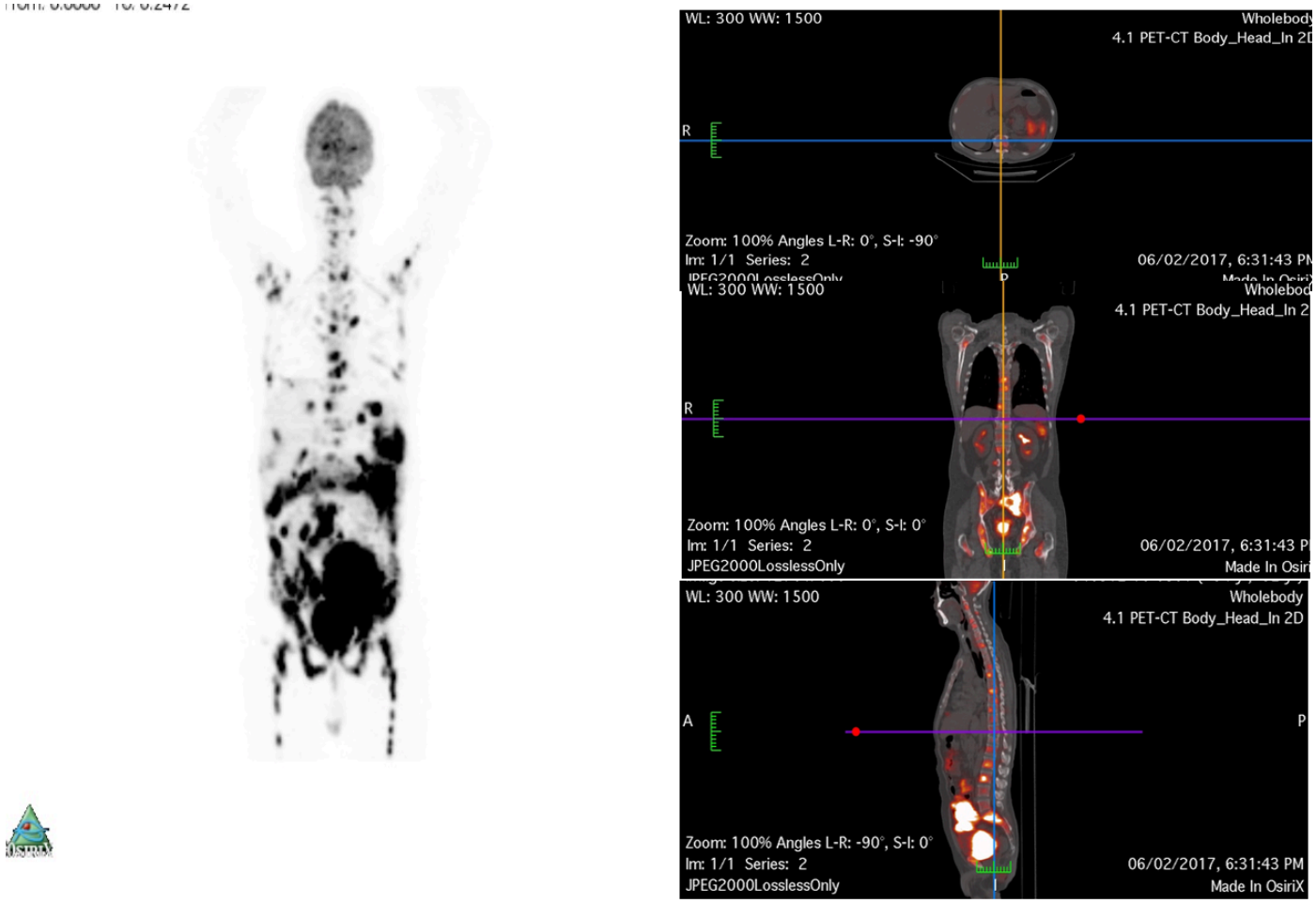

Figure 3.1. Multifocal type of uptake