

Role of Positron Emission Tomography with Computed Tomography using F-18 Flurodeoxyglucose in detection of Carcinoma of Unknown Primary in Malaysia

By

DR NURSAZLINDA BINTI SAMSUDIN

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DECLARATION

“I hereby declare that the dissertation entitled **Role of Positron Emission Tomography with Computed Tomography using F-18 Flurodeoxyglucose in detection of Carcinoma of Unknown Primary in Malaysia** is a result of my own work, except for the work that have been cited clearly in the references”

Signature:

Name: Dr Nursazlinda binti Samsudin

Date:

SUPERVISOR’S RECOGNITION

This Dissertation Report has been read and certified by

Signature:

Name: Dr Siti Zarina binti Amir Hasan

Date:

Signature:

Name: Dr Mahayudin b Abdul Manap

Date:

DEDICATION

Specially dedicated to

Dr Hj Samsudin b Hj Omar

Hjh Zainab bt Mohammed Hashim

Siti Hawa binti Boon Bujang

Nurhazlin Umairah binti Abdul Rashid

Muhammad Umar bin Abdul Rashid

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LIST OF ABBREVIATIONS AND SYMBOLS

CUP	Carcinoma of unknown primary
FDG	Fluoro-18 Flurodeoxyglucose
PET	Positron Emission Tomography
CT	Computed Tomography
PET-CT	Positron Emission Tomography with Computed Tomography
IHC	Immunohistochemistry
Rt-PCR	Real time Polymerase Chain Reaction
ToO	Tissue of Origin
CTID	Cancer Type ID
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
miRNA	MicroRNA
MRI	Magnetic Resonance Imaging
ENT	Ear Nose Throat
GLUT	Glucose Transporter
TP	True Positive

FP	False Positive
TN	True Negative
FN	False Negative

ABSTRACT

Carcinoma of unknown primary represents a heterogenous group of tumour with well known characteristics and behavior. It is essential to locate the primary tumor to facilitate specific treatment for patients. It is said that conventional investigations may detect the primary in about 30% of patients diagnosed with this condition. F-18- flurodeoxyglucose PET-CT has been used to detect the primary tumor when conventional methods fail. This study retrospectively investigates the role of F-18-flurodeoxyglucose in detection of primary in Carcinoma of Unknown Primary in Malaysia. We found that F-18-flurodeoxyglucose PET-CT localised the primary in 40% of patients, has a sensitivity of 76.67%, specificity of 92.11% and positive predictive value of 88.46%. The most common site of the primary is the lung, followed by the ovaries and the pancreas.

ABSTRAK

Carcinoma of unknown primary adalah satu kumpulan pelbagai tumor dengan sifat dan tingkahl aku yang telah diketahui. Adalah menjadi satu keperluan untuk mengesan lokasi utama kanser tersebut bagi memudahkan pemberian rawatan yang lebih berkesan dan khusus untuk pesakit. Kajian yang telah dijalankan sebelum ini telah menunjukkan bahawa siasatan konvensional boleh mengesan kedudukan barah primer dalam kira-kira 30% daripada pesakit yang didiagnosis dengan keadaan ini. FDG PET-CT adalah salah satu lagi cara untuk mengesan barah apabila kaedah konvensional gagal. Kertas ini secara retrospektif menyiasat peranan FDG PET-CT dalam mengesan *Carcinoma of unknown primary* di Malaysia. Kami mendapati lokasi primer barah berhasil dikesan oleh FDG PET/CT di dalam 40 % daripada pesakit yang telah dirujuk. Ia adalah 76.67 % sensitif, mempunyai kekhususan 92.11 % dan mempunyai nilai ramalan positif sebanyak 88.46 %. Lokasi utama yang kerap di diagnosa adalah adalah paru-paru , diikuti oleh ovari dan pankreas.

CHAPTER ONE

INTRODUCTION

Carcinoma of Unknown Primary or commonly known as CUP is a heterogeneous group of tumor which has metastasized from its primary site at the time of diagnosis. It occurs in 3-5% of patients diagnosed with cancer (Pavlidis et al 2003). Diagnosis is only made after tissue biopsy confirms the metastatic disease where conventional radiology, biochemical tumor markers, immunohistochemistry and endoscopies fail to identify the primary site, which the cancer originates from.

The identification of primary site of the tumor is of importance, as this will ensure definitive treatment to be given in tailoring treatment for patients. Detection and appropriate staging can often change management (Pelosi et al., 2006) and the course of disease (Raber et al., 1991 and Lenzi et al., 1997). Hence, identification of the primary site and staging is pertinent to further tailor treatment thus determining the management of patients, eventually determine the prognosis of patients.

Majority of patients diagnosed with CUP has poor prognosis. Median survival for patients diagnosed with CUP said to be between 4 to 12 months (Altman et al., 1986, Raber et al., 1991, Lenzi et al., 1997 and Chorost et al. 2004). This is due to the tumour behaviour and also due to

the fact that majority will not respond to treatment. Half of the patients will succumb to the disease after 1 year and much less than that will be surviving 5 years after being diagnosed. Systemic chemotherapy may prolong survival up to 24 months, but it comes with severe side effect such as grade III/ IV myelosuppression (Raber et al., 1991).

Prior to being diagnosed with CUP, patients will be subjected to a lot of investigations in order to determine the primary. These are often costly and time consuming. Conventional investigations is said to identify the primary tumor in 27-40% of patients diagnosed with CUP (Le Chevalier et al., 1988 and Abbruzzese et al., 2009). However, even at autopsy about 50% of the primary sites are not identified (Le Chevalier et al. 1988 and Al-Brahim et al., 2005). As time is of the essence for patients diagnosed with CUP, it is hoped that there is a diagnostic tool sensitive and specific that can be utilised to elicit the primary tumor.

The use of positron emission tomography, PET coupled with computed tomography, CT utilizing F-18 Fluorodeoxyglucose, FDG a radioactive glucose analogue allows the evaluation of metabolic activity of cells instead of anatomical abnormalities detected by conventional radiology. This study is aimed to evaluate the role of positron emission tomography combined with computed tomography using F-18 Fluorodeoxyglucose in patients diagnosed with carcinoma of unknown primary in Malaysia.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of carcinoma of unknown primary

Carcinoma of unknown primary, CUP is a group of heterogeneous tumor but with similar biological behaviour. Patients often present with variety of presentation, which is usually related to the metastatic site. Multiple sites or organs involvements are seen in more than 30-50% of these patients (Abbruzzese et al. 1994 and Briasoulis et al. 1997). Patients are often diagnosed with CUP after conventional radiology, biochemical tumor markers, immunohistochemistry and endoscopies are unsuccessful in attaining the tumor origin following tissue biopsy from the metastatic site.

2.2 Epidemiology

CUP accounts for 2.3% to 4.2% of all human cancer. It is the seventh to eight most common type of cancer and the fourth frequent cause of cancer related death, both in male and female in the world (Pavlidis and Fizazi, 2005). The overall age-standardised incidence per 100 000 people per year is 7-12 cases in USA and it occurs in 3-5% of all newly diagnosed cancer patients (Pavlidis and Fizazi, 2005). At presentation, median age is approximately 60 with a slight higher incidence in male (Pavlidis et al., 2003). In Malaysia, CUP accounts for 3.4% of newly diagnosed cancer in males and 1.9 % in females (Omar and Tamin, 2011). According to the same report, the

highest incidence of CUP was observed in the 65 - 69 age group for males and in the 55 - 59 age group for females. CUP is the 9th most common cancer in males and the 13th most common cancer in females in Malaysia. The incidence is higher in males (59.4%) compared to female (40.6%) with slightly higher preponderance in males of Malay ethnicity (25%) compared to other races.

2.3 Clinical and biological characteristics

Carcinomas of unknown primary tumours share similar clinical and biological characteristics. Non-specific complaints within a short period of time for example loss of weight and loss of appetite are common presentation in patients diagnosed with CUP. The location of the primary origin may remain undiscovered during the patient's existence, even after autopsy only 70-80% of primary tumours is reported to be found (Le Chavlier et al. 1988). The similar study also noted unusual pattern of metastases, frequently involving the kidneys, adrenal gland, skin and heart. Another interesting observation that they made was, even at autopsy there were differences in metastatic localization between those of CUP and known primary tumours. Many factors may contribute to this elusiveness including its small size and sub mucosal location. Another hypothesis is that the angiogenic incompetence of the primary tumor leads to marked apoptosis and cell turnover (Naresh, K.N., 2002). CUP can be categorised into four major subtypes, with adenocarcinomas of well or moderate differentiation the most predominantly histological finding in 60% of cases (Pavlidis et al 2004). This was followed by undifferentiated or poorly differentiated carcinomas (30%), squamous cell carcinoma (5%) and undifferentiated carcinoma (5%).

2.4 Diagnostic evaluation of carcinoma of unknown primary

Patients with CUP are often subjected to undergo pathology, biochemistry, imaging and endoscopic studies for diagnostic evaluation. Adequate tissue sample is needed in order to perform light microscopy, immunohistochemistry, IHC and markers/ receptor studies as well as more specific investigations such as electron microscopy or genetic analysis. Light microscopic examination with staining, may characterise cell morphology and cell differentiation. On light microscopy, most CUP cancers are identified as adenocarcinoma (60%) or poorly differentiated adenocarcinoma or undifferentiated carcinoma or neoplasm (30%–35%); the remaining lesions are squamous cell carcinoma or neuroendocrine cancers (5%) (Varadhachary, G.R., 2007)

Further characterization of the carcinoma can be done with immunohistochemistry, IHC studies. IHC stains are peroxidase labelled antibodies against specific tumor antigens that are used to define tumor lineage. Meta-analysis of 5 studies by Anderson and Weiss in 2010 showed IHC studies correctly identified the tissues in 82.3% (95%, confidence interval 77.4 to 86.3%) when both the primary and metastatic tumour were studied together. This being said, the same study also found that when the metastatic tumor was studied without knowing the primary tumor, IHC studies only correctly identified the tissues in 65.5% (95% confidence interval 60.1-70.7%) of cases. IHC screening is also costly and time consuming, which requires special expertise and may not be available in all institutions (Oien & Dennis , 2012).

Another method used to identify the primary origin of CUP is by performing molecular profiling of the biopsied tissue. This can be performed with minimal tissue biopsy, which is usually the case in samples acquired from fine needle biopsies. DNA or RNA (messenger RNA (mRNA) or microRNA (miRNA)) microarray or quantitative real-time polymerase-chain-reaction (rt-PCCR) assays are often used to ascertain the primary location of the tumor. In these methods, the genes from CUP are evaluated and assumptions are made that the molecular signals of the metastatic tumours matches their primary origin. Hence by matching molecular signals, the primary tumor could be identified. There are currently three mRNA and miRNA methods, which are available commercially, used in order to ascertain the origin of the tumor in CUP. The Pathwork Tissue of Origin, TOO test is a microarray-based gene expression assay assessment which analyses 2000 mRNAs of the biopsy tissue (Pillai et al 2011). It has a database of 2140 tumor of 58 types and subtypes grouped 15 classes which includes bladder, breast, colorectal, gastric, germ cell, hepatocellular, kidney, non-small cell lung, non-Hodgkin lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid. The ToO test will report on the similarity scores, SS comparing the above mentioned 15 tumor classes. The higher the SS, the more likely the diagnosis is. BioTheranostic's Cancer Type ID, (CTID) uses the rt-PCR method to look at 92mRNAs. It has a database, which contains 2206 tumours of 30 main types and 54 subtypes (Erlander et al, 2011). Whilst the miRview mets2 looks at 64miRNAs by microarray with a dataset which contains 1282 tumours with 42 types and subtypes (Meiri E et al, 2012). Despite very high specificity of these three molecular profiling methods, their sensitivity in known origin ranges from 72% to 95% and sensitivity is often lower in metastases than in primary tumours (Takei et al, 2011). Study done by Pillai et al (2011) found that the TOO test showed 91% sensitivity in 179 known metastases and 87% sensitivity in 283 poorly differentiated or

undifferentiated primary tumours. CTID showed 83% sensitivity in 187 known primary tumours (Erlander et al, 2011). Kerr et al (2012) found that CTID showed sensitivities of 87% for tumor typing and 82% for subtyping in 790 known primary and metastatic cancers in an independent study. Meiri et al (2012) showed that mirView mets2 showed 85% sensitivity in 509 known primary and metastatic tumours. However, when applied to CUP, the ToO test generated a prediction in 96% of 45 samples, which was found clinically appropriate (Hainsworth et al, 2011). Meanwhile, CTID yielded a prediction in 91% of 815 submitted cancers of indeterminate or unknown primary (Schroeder et al, 2012). In another study by Greco et al (2010), CTID provided 20 predictions in patients of CUP patients whom the primary site became unknown. According to the same study, 75% of the predictions were correct, 10% were indeterminate and 15% appeared incorrect. miRview mets2 agreed with clinic-pathological data in 88% of 55 brain CUPs (Meiri et al 2012) and in another study of 84 CUP patients, the test agreed in 92% of 84 assessable cases (Pentheroudakis et al, 2013). However, there are limits to what molecular profiling has to offer. The ToO test for example, as its result is reported as one of 15 classes, the tumor subtype at a given site does not appear to be described and also the classes lacks certain tumor types which include neuroendocrine tumours, cholangiocarcinoma and mesothelioma (Pillai et al, 2011). In all three tests, common incorrect diagnoses include pancreatic, colonic and gastroesophageal cancer (Greco et al 2010 and Kerr et al 2012). Added to the above-mentioned problems with molecular profiling, the mentioned commercially available kits are very costly. ToO testing costs between 3000 and 4000 US dollars, or roughly 10 to 20 times the cost of immunohistochemistry (Oien & Dennis, 2012)

Routine use of serum tumor marker is not recommended as it has not been proven to be of prognostic value and will not assist in the diagnosis of the origin of the primary site. This was attributed to over expression of tumor markers in patients with CUP (Milovic et al. 2002). However, there is serum markers recommended for different subgroups of patients which has found to be of benefit. Serum markers recommended for males presenting with CUP are serum β -HCG, AFP and PSA to exclude treatable extra-gonadal germ cell tumours and identify metastatic prostate carcinoma amendable to endocrine treatment (Pavlidis et al. 2003). Serum thyroglobulin is also recommended in patients presenting with bone metastases to rule out thyroid carcinoma. Serum Ca 15-3 and Ca 125 are said to be useful in patients presenting with axillary node adenocarcinomas and in peritoneal papillary adenocarcinomatosis (Milovic et al. 2002)

The choice of imaging investigations performed is based on the clinical presentation of these patients. It is said that the primary tumours are detected by conventional imaging (Nanni et al. 2005) in up to 27% of cases. Ultrasound is a quick, easy, involves no radiation and can be done at the patients' bedside. It is nevertheless, operator dependent causing variation in findings. Chest x-ray is very simple and routinely done. However, a cross sectional whole body imaging is desired as the primary site of the tumor can be anywhere in the body. Computed Tomography, CT scan is another imaging modality that is routinely performed to assist in the diagnosis. Chest, abdominal and pelvic CT scan may be performed depending on the clinical presentation of patients. Not only CT scan may help detect the primary tumor site, it can also assess the stage of the disease and at the same setting locate the site that can be biopsied. CT of the abdomen and

pelvis results in the detection of a primary site for the cancer in 30–35% of patients (Karsell et al. 1982). Female patient who presents with metastatic adenocarcinoma and clinical presentation suggestive of breast carcinoma may undergo ultrasound of the breast and mammogram. MRI breast can also be utilized in these patients if ultrasound and mammogram shows negative results (Olson et al., 2000). However, CT and MRI can miss small lesion or pathological changes in normally sized tissues especially in CUP cases where the primary tumours are said to be small (Pavlidis 2007).

Endoscopic investigations are used to evaluate patients with specific clinical presentation. It is recommended that patients presenting with solitary cervical node to have ENT endoscopy, patients with thoracic indications or pulmonary symptoms should undergo fibre optic bronchoscopy, patients with abdominal symptoms or with positive faecal occult blood test should have gastrointestinal endoscopies in and patients with inguinal lymph node involvement should have proctoscopy and/ or colposcopy (Fizazi et al 2015 & Casciato et al., 1990).

2.5 Treatment of carcinoma of unknown primary

Besides than identifying the location of the primary, patients can also be classified into favourable and unfavourable subsets. This is done based on clinical and pathology criteria, whereby patients with CUP can be divided into favourable and unfavourable prognosis (Palvadis et al 2003). Favourable prognosis includes women with peritoneal carcinomatosis, women with isolated axillary lymph node metastases, adenocarcinoma presenting as single metastatic lesion,

young men with features of extra gonadal germ cell tumor, squamous cell involving the cervical or inguinal lymph nodes and neuroendocrine carcinoma (Hainworth JD and Fizazi K, 2009). This being said, only 15-20% of patients fall into the favourable prognosis and the majority will fall into the unfavourable prognosis. The unfavourable prognosis patients, unlike the favourable prognosis patients do not have tumours that are chemo sensitive and may not experience long-term disease control. Despite management with a variety of chemotherapeutic combination, patients with unfavourable prognosis (especially those with poor performance status and abnormal serum lactate dehydrogenase) have poor prognosis (Golfinopoulos et al 2009).

Systemic chemotherapy is the main treatment modality for most patients, but surgery, radiation therapy, and even periods of observation are important. The regimes, which are recommended based on thorough evaluation, are the combination of taxane/ platinum or gemcitabine/ platinum. The response rate is said to be 30 to 40% with median survivals of 8 to 11 months (Culine et al, 2003 & Hubner et al, 2009).

2.6 Carcinoma of unknown primary and F-18 FDG PET

As previously mentioned, one of the characteristic of CUP is its early dissemination and its aggressiveness. Primary tumours with metastatic ability, most often have increased glucose metabolism due to its rapid growth. F-18 Flurodeoxyglucose, FDG is a radioactive glucose analogue which unlike conventional radiology, looks at the tissue metabolism. It will be taken up by cells by facilitated passive diffusion through the glucose transporters, GLUT and

phosphorylated by hexokinase intracellularly. Once entering the cells, F-18 FDG will be trapped, as it will not be metabolized like normal glucose. There are 5 subtypes of GLUT (GLUT-1 to GLUT-5) and of these; GLUT-1 is found to be over expressed in tumor cells. Hexokinase is also over expressed causing higher F-18 FDG trapping in the tumor cell. F18-FDG will undergo spontaneous decay and emit positron particles which will be annihilated by electrons to produce two photons with the energy of 511keV directed 180° apart. These photons will be detected by the crystals of the PET camera, resulting in images representing the bio distribution of F-18 FDG.

Since the introduction of FDG and Positron Emission Tomography, PET several studies have been carried out to evaluate the role of FDG PET in the detection of primary location of the tumor in patients diagnosed with CUP. Delgado et al. (2003) performed a meta-analysis, which showed that the primary tumor was identified in 43% of patients with CUP referred for FDG PET study. The same study also found sensitivity and specificity of F-18 FDG PET in detection of the primary site was 87% and 71%. Another systemic review done by Sève et al. (2006) found that F-18 FDG detected the primary tumor in 41% of patients diagnosed with CUP and that F-18 FDG changed the oncology management in approximately one-third of the patients in the studies. The role of F-18 FDG PET has been established in management of metastatic squamous carcinoma of the cervical nodes. The primary tumor was ascertained in one third of patients diagnosed with CUP, presenting with this histology from biopsy of the cervical node (Bohuslavizki et al. 2000). In addition, its also found that F-18 FDG PET might influence the

treatment for patients in this group with localized node involvement, which will be considered for local treatment (external beam radiotherapy) instead of systemic chemotherapy.

2.7 Carcinoma of unknown primary and F-18 FDG PET-CT

Similar to any other diagnostic tool, FDG PET is not without its limitation. One of the problems encountered previously was localization of lesion seen on imaging, as the spatial resolution of PET camera detectors was very poor, it may contribute to false negative result. With the introduction of hybrid imaging, which uses CT scan to correctly locate the lesion with high FDG uptake, localization of lesions improved (Keller et al 2011) from 31% to 55%. Hence, hybrid imaging or PET-CT is now the standard current practise rather than PET alone. In a study by Pelosi et al 2006, the primary tumor site was detected in correctly identified in 24 out of 68 patients (35.3%). In the similar study (Pelosi et al 2006) also showed that the identification rate of the primary site is higher in the presence of adenocarcinoma compared to carcinoma histotype and that FDG PET-CT influenced the treatment instituted by oncology team in almost 50% of the cases. Other causes of false negative result are tumor with low metabolic rate and tumor with known lower avidity for FDG. False positive findings in PET-CT utilising FDG are commonly seen in infection, when there is increased utilization of glucose by granulocytes and monocytes during their metabolic burst. Inflammation, high physiological glucose uptake in normal tissue and regions of discrete uptake can also contribute to false positive results.

CHAPTER THREE

OBJECTIVES AND HYPOTHESIS

3.1 General Objective

To investigate the role of Positron Emission Tomography with Computed Tomography, PET-CT using F-18 Flurodeoxyglucose, FDG in detection of Carcinoma of Unknown Primary in Malaysia.

3.2 Specific objective

1. To identify the relationship between metastatic biopsy (metastatic adenocarcinoma and metastatic carcinoma) histopathology and identification rate of the primary tumour.
2. To identify the relationship between metastatic location (cervical and extra cervical) and identification rate of the primary tumour.
3. To evaluate the identification rate of primary tumour in patients with carcinoma of unknown primary with PET-CT using F-18 FDG.
4. To calculate the sensitivity, specificity and positive predictive value of F-18 FDG PET-CT in detecting the primary tumour sites of patients diagnosed with carcinoma of unknown primary.

3.3 Research hypothesis

1. There is a difference between identification rates of metastatic adenocarcinoma and metastatic carcinoma
2. There is a difference between identification rates of cervical and extra cervical metastases.

3.4 Null hypothesis

1. There is no difference between identification of primary tumour rates of metastatic adenocarcinoma and metastatic carcinoma
2. There is no difference between identification rates of cervical and extra cervical metastases.

3.5 Rationale of study

In Malaysia, PET-CT using F-18 FDG has been used as a diagnostic tool to search for primary site for CUP when other modalities fail. There is so far no study done to assess the role of PET-CT in detection of CUP in Malaysia. PET-CT not only has show to play a role in diagnosis of carcinoma of unknown primary, it can also be used to assess for distant metastasis hence can contribute towards the management of patients diagnosed with CUP.

3.5 Benefits of the study

1. Benefit to patient as early diagnosis of primary site, limits unnecessary investigation, hence saves time and cost.

2. Benefit to physician as tailored treatment can be instituted to patient as soon as possible, changes prognosis and can be used to assess treatment in patients.

CHAPTER FOUR

METHODOLOGY

4.1 Subjects and material

This is a retrospective study, which was carried out in the Department of Nuclear Medicine, National Cancer Institute, Putrajaya, Malaysia. The subjects are patients who were referred from other tertiary hospitals from all over Malaysia. Patients who were included in this study are those who met the following inclusion criteria: (1) Positive histology findings from metastases from unknown primary site. (2) Patients who have undergone a thorough diagnostic evaluation by the referring team which included radiological and IHC investigation. (3) No prior anti-cancer treatment done. (4) No history of previous malignancy. The standard operative procedures of the department were used to conduct the PET-CT acquisition. Data collection was carried out from the FDG PET/CT database and patient's medical records, and then stored in a secured computer. Ethical clearance from Medical Research and Ethics Committee (MREC) Malaysia as well as Jawatankuasa Etika Penyelidikan (Manusia) JEPeM, USM were obtained prior to the commencement of this study. This study was carried out according to the Declaration of Helsinki. Informed consent was waived due to the retrospective design of this study.

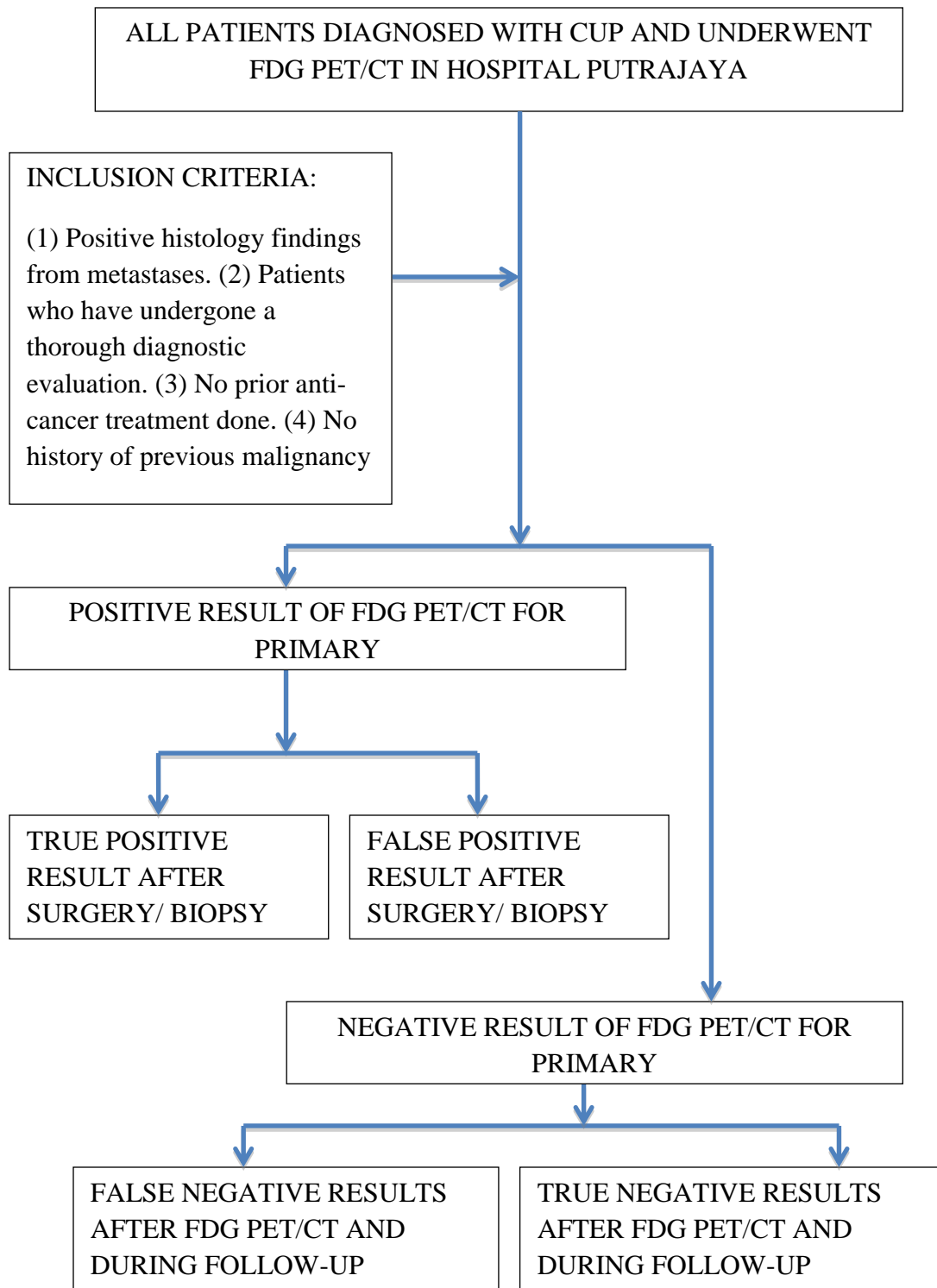


Figure 4.1: Flow chart of study protocol.

4.2 F-18 FDG PET-CT Imaging

Patients were advised to fast for at least 6 hours and abstain from strenuous exercise for 24 hours prior to the PET-CT appointment day. Capillary blood was used to assess fasting glucose levels. The preferred glucose level was $< 8.3\text{mmol/L}$, if exceeded, subcutaneous insulin was administered. F-18 FDG injections were only given 2 hours after administration of subcutaneous insulin. If the fasting glucose level exceeded 11.0mmol/L , the PET-CT will be differed and patients were referred back for glucose control. Patients with fasting glucose level of 11.0mmol/L and bellow received 5MBq/kg of F-18 FDG, which was administered intravenously by experienced nurses who are trained to deliver F-18 FDG. This was followed by 45 minutes to 1 hour of resting in an ambience room. Imaging was carried out without intravenous or oral CT contrast after 45 minutes to 1 hour of FDG injection.

An integrated PET-CT system (Discovery ST, GE Medical System, Milwaukee, USA) was used for all the patients who underwent the F-18 FDG PET-CT for this study. The image acquisition was started with CT scan from the head to the mid-thigh region (60mA , 140kV , tube-rotation time of 0.8 second per rotation) during current breathing. Immediately followed by PET (two-dimensional) from the head to the mid-thighs as well (4 minutes emission scan per table position, 3.3mm slice thickness with 7 fields of view and 15cm per field). Filtered back-projection and ordered-subset expectation-maximization (OS-EM) (two iterations, 15 subsets and matrix of 128×128) were used to reconstruct the PET data with and without CT attenuation correction. A dedicated workstation running Advantage version 4.2 software (GE Medical System, Milwaukee, USA) was used to display the attenuation corrected PET, CT and combined PET-CT

images. The results were reviewed by two experienced nuclear medicine physicians sequentially and consensus reporting was made. Two methods applied, which were visual assessment and semi quantitative measurement of FDG uptake using maximum standardized uptake values (SUVmax) to interpret the PET-CT scans.

The final result was confirmed with analysis of histologic confirmative examination and/or formal clinical follow-up findings were served as the reference standard. Formal clinical follow-up includes physical examination and radiological imaging. FDG PET-CT positive results were defined as true positive, TP when confirmed by the reference standard and as false positive, FP when the reference standard revealed no evidence of PET-CT results. True negative is defined by negative FDG PET-CT and confirmed by the reference standard, false negative, FN when there was subsequent proof of malignancy by the reference standard despite negative FDG PET-CT.

4.3 Statistical Analysis

Statistical analysis was performed using SPSS Version 22.0 to assess whether there was correlations between findings of the PET-CT scan with the tumour histopathology examination and between PET-CT findings with groups of patients with cervical and extra cervical lymph node metastases. The p-values less than 0.05 were considered statistically significant. Sensitivity, specificity and positive predictive values were calculated using standard statistical formula. Formula for calculating diagnostic FDG PET-CT in detecting primary tumor were as follow: sensitivity = $TP/(TP+FN)$, specificity = $TN/(TN+FP)$, positive predictive value, PPV = $TP/(TP+FP)$

.

CHAPTER FIVE

RESULTS

5.1 Patient Demographics

A total of 112 patients were diagnosed with CUP and underwent FDG PET-CT from 1st July 2013 and 31st December 2014. However, only 70 patients fulfilled the inclusion criteria of this study and were recruited. Thirty-one patients (44.3%) were male and the remaining thirty-nine (55.7%) were female, giving the male to female ratio of 1:1.8. The median age of the study sample was 56 years old (SD 14.11). Half of the study samples were Malay, followed by Chinese and Indian with 32.9% and 17.1 % respectively (**TABLE 5.1**).

42 out of 70 (60%) patients enrolled, had biopsy, which showed carcinoma histopathology (20 metastatic carcinoma, 9 poorly differentiated carcinoma, 8 metastatic squamous carcinoma, 3 metastatic papillary carcinoma and 2 undifferentiated carcinoma), 26 (37.1%) showed adenocarcinoma and 2 (2.9%) showed melanoma histopathology. Median follow up duration was 6 months (range 3-12 months).

TABLE 5.1: The demographic characteristics of study samples and the distribution of disease by HPE results.

Characteristics		Number of sample (n)	Percentage (%)
Gender:			
Male		31	44.3
Female		39	55.7
Race:			
Malay		35	50.0
Chinese		23	32.0
Indian		12	17.1
Others		0	0
Age:			
Min	15 y.o		
Max	83 y.o		
Median age	56 y.o		
SD	14.11		
Histopathology result (HPE):			
Metastatic adenocarcinoma		26	37.1
Metastatic carcinoma		42	60.0
Malignant melanoma		2	2.9