UNIVERSITI PUTRA MALAYSIA

CHOLINE AS A MOLECULAR IMAGING BIOMARKER FOR ASSESSING BREAST CANCER AGGRESSIVENESS

SHAZREEN BINTI SHAHARUDDIN

FPSK(P) 2017 36

CHOLINE AS A MOLECULAR IMAGING BIOMARKER FOR ASSESSING BREAST CANCER AGGRESSIVENESS

By

SHAZREEN BINTI SHAHARUDDIN

Thesis Submitted to the School of Graduate Studies, Universti Putra Malaysia, in Fulfillments of the Requirements for the Degree of Doctor of Philosophy

October 2017

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CHOLINE AS A MOLECULAR IMAGING BIOMARKER FOR ASSESSING BREAST CANCER AGGRESSIVENESS

By

SHAZREEN SHAHARUDDIN

October 2017

Chairman : Associate Professor Fathinul Fikri Ahmad Saad, PhD
Faculty : Medicine and Health Sciences

The level of choline kinase in human breast cancer cells was found to be overexpress as compared to normal human mammary epithelial cells. In this study we investigated the potential expression of choline in ^{18}F-FCH PET/CT as compared to F^{18}Fluorodeoxyglucose PET/CT and correlations with expression of miRNA, CD47 and histo-immunochemical markers of estrogen, progesterone and HER2 in detecting aggressiveness of breast cancer.

Twenty one patients with Birads 4 or 5 on mammogram and recurrent breast carcinoma underwent imaging of ^{18}F-Fluorocholine and ^{18}F-Fluorodeoxyglucose PET/CT. Tissue biopsy and histo-immunochemical results with blood sampling of expression of miRNA-21,miRNA-155 and CD47 were recorded. The data were evaluated by experienced nuclear imaging using the biopsy and histo-immunochemical findings as a gold standard.

There were 21 females with a mean age of 52.82 ± 10.71 years. There is a significant evidence of high uptake of ^{18}F-Fluorocholine PET/CT in the metastatic lesions (2.27±3.19) as compare to ^{18}F-Fluorodeoxyglucose (1.74±2.32, p=0.004).While there is a significant association between high ^{18}F-Fluorocholine uptake (3.53±3.51,p=0.005) with HER2-ve (1.98±2.14, p=0.009) in metastatic lesions and lymph node. High uptake of ^{18}F-Fluorocholine showed a significant correlation with expression of miRNA-21 in lymph node (1.81±2.21,p=0.05) and metastasis lesions (3.33±3.61,p=0.02). There is also a significant correlation between high choline uptake with expression of miRNA-155 (1.47±1.99,p=0.01) and CD47 (0.85±0.23,p=0.008) in lymph node and ^{18}F-Fluorocholine showed high sensitivity and specificity (40%,68.8%) compared to ^{18}F-Fluorodeoxyglucose (27.3%,60%). There is a significant association miRNA-155 between patients with recurrent breast cancer and non recurrent breast cancer (p=0.026) and expression of HER2 –ve (p=0.042).

We concluded that higher choline uptake in ^{18}F-Fluorocholine would potentially be used as novel surrogate marker for detection and prediction of an aggressive breast cancer with strong correlation with the protein overexpression of miRNA-155, miRNA-21,CD47 and HER2-ve.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

CHOLINE SEBAGAI PENANDA MOLEKULAR DALAM MENDETEKSI KANSER PAYUDARA YANG AGRESIF

Oleh

SHAZREEN SHAHARUDDIN

Oktober 2017

Pengerusi : Profesor Madya Fathinul Fikri Ahmad Saad, PhD
Fakulti : Perubatan dan Kesihatan

Tahap choline kinase dalam sel kanser payudara manusia didapati lebih tinggi berbanding dalam sel epitelium kelenjar payudara manusia biasa. Dalam kajian ini, kita menkaji potensi ^{18}F-Fluorocholine berbanding ^{18}F-Fluorodeoxyglucose dan korelasi dengan histologi payudara seperti estrogen, progesteron,HER2 dan ekspresi miRNA dan CD47 dalam mengesan kanser payudara.

Dua puluh satu pesakit yang mempunyai kanser payudara utama atau berulang menjalani pemeriksaan ^{18}F-Fluorocholine dan ^{18}F-Fluorodeoxyglucose PET/CT scan. Semua pesakit mempunyai keputusan mammogram Birads 4 atau 5. Hasil keputusan biopsi dan histologi (estrogen, progesteron dan HER2) telah direkodkan. Selain itu ekspresi miRNA-21, miRNA-155 dan CD47 juga telah direkodkan. Analisis kualitatif dibaca oleh pakar nuklear yang berpengalaman menggunakan penemuan biopsi sebagai standard utama.

Terdapat 21 pesakit dengan purata umur 52.82 ± 10.71 tahun. Terdapat signifikasi antara peningkatan ^{18}F-Fluorocholine (2.27 ± 3.19) berbanding peningkatan ^{18}F-Fluorodeoxyglucose (1.74 ± 2.32), p = 0.004.Selain itu,terdapat juga signifikasi antara peningkatan ^{18}F-Fluorocholine dengan HER2 -ve dalam nodula limfa (1.98 ± 2.14, p = 0.009) dan metastasis (3.53 ± 3.51, p = 0.005). Malah peningkatan pengambilan ^{18}F-Fluorocholine juga menunjukkan signifikasi dengan ekspresi miRNA-21 dalam nodula limfa (1.81 ± 2.21 , p = 0.05) dan metastasis (3.33 ± 3.61, p = 0.02), miRNA-155 dalam nodula limfa (1.47 ± 1.99, p = 0.01) dan CD47 dalam nodula limfa (0.85 ± 0.23) . ^{18}F-Fluorocholine juga menunjukkan kepekaan dan sensitifasi yang tinggi (40%, 68.8%) berbanding ^{18}F-Fluorodeoxyglucose (27.3%, 60%). Terdapat juga signifikasi antara ekspresi miRNA-155 dengan penyakit kanser payudara berulang dan bebas dari kanser payudara berulang (p=0,026) dan ekspresi HER2-ve (p = 0.042).

Dengan ini,kami membuat kesimpulan bahawa peningkatan choline dalam ^{18}F-Fluorocholine menujukkan bahawa radioaktif ini berpotensi dalam mendeteksi kanser payudara yang agresif dengan korelasi yang kuat dengan ekspresi protein penanda genetik seperti miRNA-155, miRNA-21 dan CD47 dan juga histoimmunokimia daripada HER2-ve.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank and praise Allah the Almighty who has enabled me to conduct and successfully completed this study. Whatever is good this work contains is due to Allah blessings and whatever is bad contains is due to myself.

I would like to express my sincere gratitude to my supervisor, Prof.Madya.Dr Fathinul Fikri Ahmad Saad for his supervision, generous support and guidance from initial to final accomplishment of this research. My warmest gratitude also goes to my co-supervisor Dr.Mohd Sohel Rana and Prof.Madya.Dr.Cheah yoke kqueen for their valuable time and insightful comments especially in helping and guide me in the statistical and genetics analysis.

My deepest appreciation is also to my external co-supervisor Dr.Ramdave Shaker for their support and assist once during my field working. This gratitude and sincere thanks to all staff of The Centre for Diagnostic Nuclear Imaging (PPDN) for their kind assistance. This work was supported by Fundamental Research Grant Scheme(FRGS) from Ministry of Higher Education, Malaysia with ID No. 28612-61021 and Research University Grants Scheme (RUGS-6) from Universiti Putra Malaysia with ID NO. GP-IPS/2016/9470600. I would like to thank to Kementerian Kesihatan Malaysia (KKM) for given permission to used the imaging machine and all the equipment for this research. I also would like to thank to Richards, Bao Jing and pathology staff for technical assistance in Biomedical laboratory and pathology laboratory, Faculty of Medicine and Health Sciences, UPM.

Above all, I shall remain eternally grateful to my beloved parents, my father Prof. Dr Shaharuddin Mohd and my mother Prof. Dr. Rosnani Hashim who will always be my enormous source of inspiration and I dedicated all my effort and hard work to both of them. To my wonderful husband Mohd Firdaus Zulkafli ,my daughter Puteri Sofia Mohd Firdaus,my sister Shazwani Shaharuddin and my brother Muhammad Shazril Shaharuddin, thank you for your support.

I certify that a Thesis Examination Committee has met on 12 October 2017 to conduct the final examination of Shazreen binti Shaharuddin on her thesis entitled "Choline as a Molecular Imaging Biomarker for Assessing Breast Cancer Aggressiveness" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Syahrilnizam bin Abdullah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Dato' Abdul Jalil bin Nordin, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Sulaiman bin Md Dom, PhD
Associate Professor
Universiti Teknologi MARA
Malaysia
(External Examiner)

Heinz-Peter Schlemmer, PhD
Professor
German Cancer Research Centre
Germany
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 30 November 2017

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor Philosophy. The members of the Supervisory Committee were as follows:

Fathinul Fikri Ahmad Saad, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairperson)

Cheah Yoke kqueen,PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Members)

Ramdave Shakher, PhD
Senior Medical Lecturer
Monash University,Australia
(Members)

Mohd Sohel Rana,PhD
Senior Lecturer
Faculty of Mathematics
Universiti Putra Malaysia
(Members)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Shazreen Binti Shaharuddin, GS43966

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Fathinul Fikri Ahmad Saad

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Cheah Yoke kqueen

Signature: _____
Name of Member of Supervisory Committee: Dr.Ramdave Shakher

Dr. Shakher has been informed on his role.

Signature: _____
Name of Member of Supervisory Committee: Dr.Mohd Sohel Rana

Dr. Sohel has been informed on his role.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BIRADS	Breast Imaging Reporting and Data System
BMI	Body mass Index
CT	Computed Tomography
Cho	Choline
[^{11}C]	Choline (CHO)
CD47	Cluster of Differentiation 47
CTC	Circulationg tumor cells
DCIS	Ductal carcinoma insitu
DMEA	Dimethyethanolamine
erbB2/HER2/her2	Human epidermal growth factor receptor 2
ER/E	Estrogen receptor
FAS	Fatty acids synthase
F -^{18} BrCH2F	F^{18}fluorobromomethane
^{18}F- FCH/ FCH	^{18}F-Fluorocholine or F-18 Fluoromethylcholine
^{18}F-FDG/FDG	^{18}F-Fluorodeoxyglucose
GMP	Good Manufacturing Practice
GPC	Glycerophosphocholine
HCC	Hepatocellular carcinoma
HU	Hounsfield Unit
IACR	International Agency for Research on Cancer
IDC	Invasive ductal carcinoma
IHC	Immunohistochemical
MM	Mammography
MIP	Multiple image projection
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectrosopy
miRNA	Micro Ribonucleic Acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
PC	Phosphatidylcholine

PE	Phosphatidylethanolamine
PET/CT	Positron Emission Tomography/Computed Tomography
PR	Progesterone
PrCR	Programmed cell removal
PPARα	Peroxisome proliferator-activated receptor alpha
ppm	Per million
PPDN	The Centre for Diagnostic Nuclear Imaging
PPUKM	Pusat Perubatan Universiti Kebangsaan Malaysia
ROI	Region of interest
SD	Standard Deviation
SIRPα	Signal-regulatory protein alpha
SPECT	Single photon emission tomography
SPSS	Statistics Package for Social Science Software
SUVmax	Standardize Uptake Value maximum
TAC	Time-activity curve
tCho	Total choline containing resonance
TG	Triglycerides
TSP-1	Thrombospondin -1
US	Ultrasound
WHO	World Health Organization.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

1.1.1 Epidemiology of Breast Cancer

According to the International Agency for Research on Cancer (IACR) Globocan of the World Health Organization (WHO) (2012), the mortality rate due to breast cancer had increased from 20,100 deaths in 2008 to 21,700 deaths in 2012. Early detection was shown to increase survival for at least five years for majority of the women. More than 90% of the women diagnosed with breast cancer managed to survive more than 20 years due to early detection and prevention (Zainal et al., 2011).

Worldwide, breast cancer is known as the main cause of death due to various factors including being overweight especially after a woman has reached menopause, prolonged hormone replacement therapy, family history of breast cancer and giving birth to a first child at an older age or have never given birth at all. Apart from these factors, changes in genetic materials such as ERBB2 may also occur as a result of exposure to infections, drugs, tobacco, chemicals and other factors. Cai-Xia et al. (2015) claimed that choline intake can cause development of breast cancer.

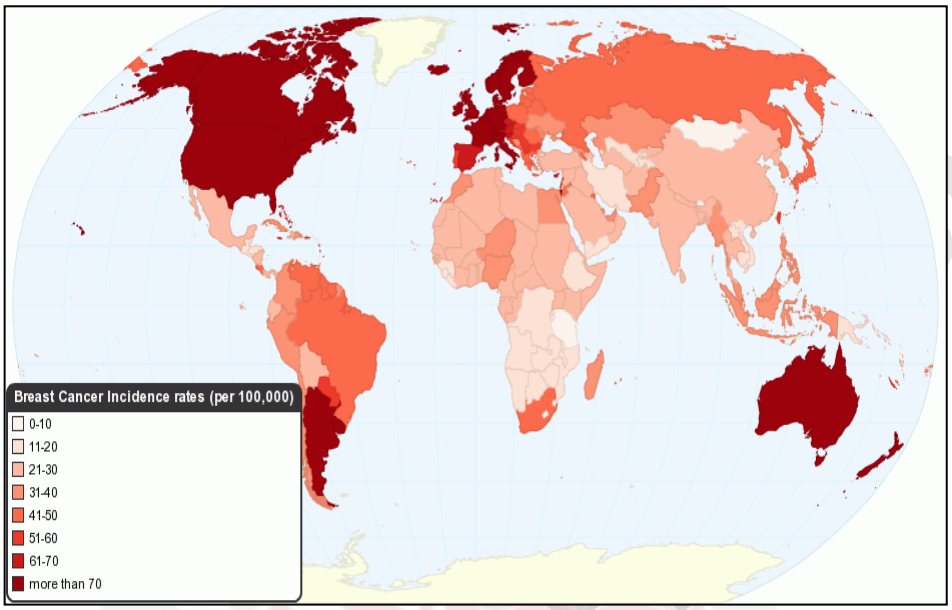

Figure 1.1: Breast Cancer Incidence and Mortality Worldwide (International Agency for Research on Cancer, 2010), GLOBOCAN 2008
(Source*:* http:www.Globocan.Iarc.Fr/Factsheets/Cancers/Breast.Asp**.)**

1.1.2 Choline in Breast Cancer

In normal human mammary epithelial cells, the level of choline was found to be much lower compared to the level found in human breast cancer cells (Aoyama et al., 2004). Zhang et al., (2013) found that the progression of breast cancer was due to the high choline intake in one's diet. The progression is caused by both choline transport and phosphorylation found to be augmented in human breast cancer cells (Ramirez et al., 2008). The development of normal human mammary epithelial cells into a malignant phenotype was shown to be associated with an overexpression of choline kinase (CK) that catalyzes the phosphorylation of choline to form phosphocholine, followed by phosphatidylcholine in the tumor cell membranes (Molina et al., 2004). Ramirez et al., (2007) showed that an increase in choline uptake in breast cells with tumor was due to upregulation of choline kinase demand of membrane constituents.

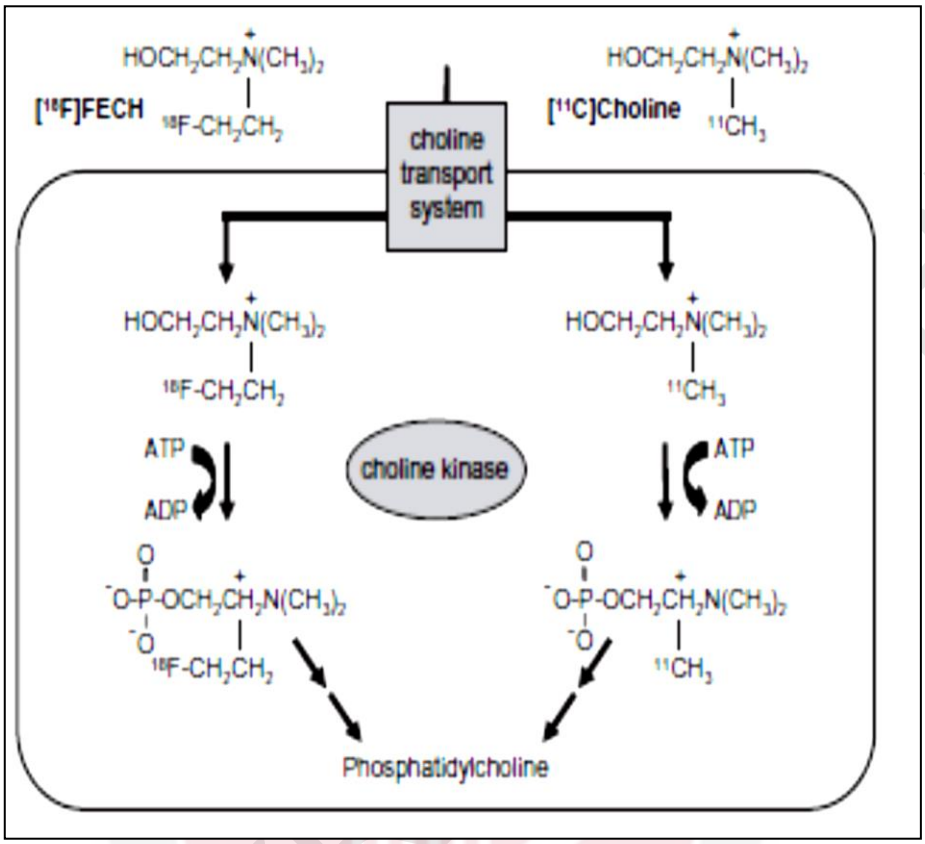

Figure 1.2 : Schematic of (^{18}F) FECH and (^{11}C) choline uptake mechanism. Both tracers are transported into cell via choline transport systems. In the cell, choline kinase catalyzes the phosphorylation of the choline to phosphorylcholine, followed by phosphatidylcholine (Glunde et al., 2011)

1.1.3 Choline and Biochemical

Previous studies showed a unique phenotype of increased choline metabolism in the transition of normal mammary epithelial cells into immortalized, oncogene-transformed and finally, nonmetastatic and metastatic cancer. In Aoyama et al.,(2004) study, a switch from predominantly higher intracellular metabolite levels of glycerophosphocholine (degradation pathway) to predominantly higher phosphocholine levels (biosynthetic pathway) early in cell transformation was observed. Phosphocholine levels then increased progressively and were at the highest levels in breast cancer cells. Further studies by Cao et al.,(2012), showed that in mammary epithelial cells also showed that aberrant increase in phosphocholine metabolite levels was due to overexpression of enzymatic choline kinase- α (Glunde et al., 2004). Cellular phosphocholine and choline kinase activity were regulated by the growth factor receptor-MAPK pathway (Choi et al., 2003), the same pathway that modulates estrogen-independent growth (Ramirez et al., 2007).

1.1.4 The Use of Choline in Human

Choline is a vital nutrient important for the development and functioning of cells, such as cell-membrane signaling (phospholipids), lipid transport (lipoproteins), neurotransmitter synthesis (acetylcholine) and methy-group metabolism (homocysteine reduction) (Penry et al., 2008). It can be acquired from a dietary source of methyl groups via biosynthesis methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) (Stead et al., 2006). Methylation reactions play an important role in the biosynthesis of lipids and regulation of metabolic pathways (Stead et al., 2006).

Choline can be synthesized through normal metabolism in human. However, the amount taken through daily diets is limited and insufficient. Most of the foods that contain choline are in the form of phosphatidylcholine, known as lecithin. They include wheat germ, broccoli, cauliflower, peanuts, milk, meat, fish, liver and egg yolks, one of the best sources. Egg is a great dietary source for normal metabolism in human as it contains 126mg of choline (Zeisel, 2006). Choline intake of at least 425mg a day for women, 450mg a day for pregnant women and 550mg a day for men and breastfeeding women are recommended by the Institute of Medicine (Li et al., 2008).

Choline intake is important for metabolism, and it helps in reducing the risk of breast cancer. A recent study showed that 24% out of 3,000 adult women showed a reduced risk of developing breast cancer with a high intake of choline compared to women with low choline intake (Xu et al., 2008). A case-control study by the Long Island Breast Cancer Study Project found that high free choline intake lowered the risk of developing breast cancer (Xu et al., 2009). In addition, higher betaine, phosphocholine and free choline intakes also reduced mortality caused by breast cancer.

The dietary choline from foods containing sources of choline are absorbed in the intestines via choline transporters (Li et al., 2008). Li et al. (2008) found that choline is recycled in the liver, and if the supply is low, it will be redistributed back to the liver and brain from the kidney, lung and intestines. Choline will immediately be phosphorylated to phosphocholine upon entry into the cells or oxidised to betaine in some cell types such as hepatocytes (Li et al., 2008). Betaine is used in the donation of methyl groups to homocysteine to form the essential amino acid methionine (Zeisel et al., 2006).

1.1.5 Molecular Imaging and Breast Cancer

Breast cancer is a common cancer in women that develops from the aggressiveness of breast tissues. The progression of breast cancer tissues is due to multifactorial factors such as genetic and hormone replacement–estrogen. Some researchers by Lee et al., (2010) have also found the link between molecular markers and lipid metabolism of the cells. In this regard, choline intake and the development of breast cancer were noted to have underpinned the proliferation of cancer cells (Chiuve at al., 2007). The level of choline was found to be higher in human breast cancer cells compared to the normal human mammary epithelial cells (Eliyahu et al., 2007). Thus, the development of human

breast cancer was proven to be associated with the overall increase in the content of choline kinase, the enzyme that phosphorylates choline to phosphocholine. Nonetheless, there were yet any substantial data that determine the feasibility of choline as a surrogate marker for breast cancer signaling in cancer diagnosis.

Tumor landscapes are varied, and depending on the orchestration of specific molecular pathways, signaling of its early manifestation could be understood. The conventional ways to signal the metabolic expression of cancers are notably via recognizing glucose intake in the cell (Eliyahu et al., 2007). The altered glycolytic pathways in the cells is when the transport of the glucose is unchecked by the high demand of the ATP production by the cancer cells notwithstanding the glucose analog (Flurodeoxyglucose-FDG) signaling in cells. However, there are other cellular mechanisms that could be explored such as cellular proliferation, amino acid metabolism and apoptosis in the cellular reprogramming which could potentially determine the aggressiveness of the cancer (Maria et al., 2014). In short, molecular imaging-based radiotracer has been proven to be useful in detecting breast cell trafficking unlike molecular-genetic and radionuclide, which have yet to be determined. Besides that, for enzyme-based imaging or for studying the pharmacokinetic of chemotherapeutic agents, the radiotracer technique predominates. This review highlights the utilization of the molecular imaging techniques in expressing the altered lipid metabolism i.e. choline transport in cells as an index of tumor aggressiveness in breast cancer.

1.1.6 Approaches to In Vivo Molecular Imaging

Nowadays, multimodality imaging techniques such as ultrasound, mammogram, MRI and metabolic imaging such as SPECT and PET/CT are used in the diagnosis of breast cancer (Dorrius et al., 2015). In particular, and by far more common, is the radiotracer imaging of ^{18}F-Fluorodeoxyglucose (FDG) PET/CT, a glucose analogue which enables a non-invasive imaging of the glycolytic metabolism. Nevertheless, the functions of ^{18}F-FDG PET in determining the response to treatment of breast cancer are deemed to poorly specify some of the histological breast cancer types (Groheux et al., 2013). The molecular imaging of FDG has shown a real limitation and enticed the search for a new radiotracer as an alternative to signal for different biological disease processes. Relying on a single tracer approach may hamper diagnostic accuracy in detecting breast cancer.

MRI spectroscopy is also widely used to detect breast cancer by using total choline (Shin et al., 2012). The degree of elevated choline-containing compounds is related to the grade of the tumor; a high level of choline indicates malignant lesions while a low level of choline indicates benign or normal breast tissues (Jacobs et al., 2010). However, pseudo lesions are sometimes detected because the uptake by benign lesions and normal mammary gland is enhanced thus, slightly reducing the specificity of breast MRI. As there are some limitations in the detection or evaluation of the extent of those small lesions which frequently show a low uptake in the PET/CT alone, a combination of PET and MRI may provide a good quality and an improvement in diagnostic performance and response of tumor to target therapy.

1.1.7 Cellular Targets and Genetic Profiling

Breast cancer develops in most women worldwide due to various morphologic features, especially from a group of molecularly distinct neoplastic disorder (Li et al., 2010). Breast carcinoma can be categorized according to the gene expression based on intrinsic classification such as luminal A, luminal B, human epidermal growth factor receptor 2 (HER2), basal-like subtype and normal breast-like. Besides these, the histologic markers of the estrogen and progesterone hormones are also useful in detecting the prognosis of breast cancer.

Several studies have proven that genetics are also responsible for the development of breast cancer. miRNAs are suitable genetic markers that act as oncogenic and tumor suppressors (Si et al., 2013). miRNA-21 is a protein genetic that plays a role in proper cell cycle progressions and regulations of apoptosis (Si et al., 2013). The expression of miRNA provides vital diagnostic information in tumor specific patterns in determining the effectiveness of prevention and treatment of cancer (Huimin et al., 2015). Mar-Aguilar et al. (2013) claimed that miRNA has the potential to be used as the biomarkers for evaluating breast cancer due to the stability of serum and plasma in human.

Recent studies by Lee et al., (2014) have found that CD47 (Cluster of Differentiation 47) expression is useful in detecting the progression of breast tumors. CD47 is a transmembrane protein involved in a range of cellular processes including proliferation, adhesion, migration and apoptosis. CD47 also plays a role in angiogenic responses by enabling cancer cells to avoid destruction by macrophages and promote further mitotic proliferation. In various human cancers, CD47 expression is required to eliminate and avoid innate immune surveillance by phagocytosis (Paola et al., 2017). In breast cancer, carcinogenicity may promote CD47-SIRPA cell signaling system in the bone marrow and in peripheral blood thus, possibly promoting the presence of cancer-specific microenvironmental areas that support micrometastasis. One study showed that critical microenvironmental stimulus in progression of breast cancer induced by expression of CD47 led to a decrease of phagocytosis of cancer cells by macrophages and induction of the breast cancer phenotype, which promotes cancer aggressiveness and mortality (Huimin et al., 2015). Nevertheless, CD47's association with choline metabolism is yet to be known.

1.2 Problem Statement and Justification

The number of breast cancer cases which is common among women is expected to rise. In 2012 alone, 1.67 million new breast cancer cases were diagnosed (Ferlay et al., 2013). Although early diagnosis is important in preventing breast cancer from worsening, detecting breast cancer is currently done through invasive procedures (i.e. biopsy and histology) and non-invasive procedures (ultrasound, mammography and magnetic resonance imaging (MRI) that have been used as diagnostic tools for several years). These conventional techniques, however, have been implicated in inaccuracies in diagnosing breast cell malignancy (Tozaki et al., 2010). The use of well FDG marker in PET-CT as a popular molecular imaging technique is also deemed to lead to poor

diagnostic dilemma. ^{18}F-FDG PET-CT is insensitive in detecting a malignant breast cell types i.e. lobular carcinoma aside from the small volume lesion which shows a low uptake of ^{18}F-FDG on PET/CT image. Since the number of breast cancer patients continues to increase over the years, a single tracer approach may hamper diagnostic accuracy in detecting breast cancer. Therefore, the search for a new and novel molecular imaging marker in signaling tumor aggressiveness on the lipid metabolism exploiting choline cellular pathway cellular could potentially become a new surrogate marker for cancer aggressiveness.

A study related to fluorocholine in breast cancer showed limited result because of the small number of subjects (Damita et al., 2011). The choline marker has by far been used to signal prostate cancer (Schwarzenbock et al., 2012) with promising roles in influencing patient management. Therefore, its role could be potentially coupled with FDG marker in improving accuracy of detection which would lead to a better prognosis of the disease by improving the selection of patient taking specific treatment plans. The correlation of these molecular imaging markers FCH and FDG is yet to reveal their association with genetic profiling. The importance of genetic markers i.e. miRNA and CD47 would serve as a strong epigenetic basis as the new surrogate marker in the expression of cells tumor and the potentials of potent correlative markers to the cell membrane and glucose metabolism cellular reprogramming (Shigemasa et al., 2012).

This study explored all these molecular imaging of choline via multiparametric imaging modalities in its effort to provide new insights on the new standard of diagnosing aggressive breast cancer cells. This study aimed at determining whether choline signaling could potentially be used as an adjunct to the FDG (glucose) signaling in the evaluation of breast cancer. The choline radiotracer of ^{18}F-Fluorocholine (FCH) for cancer with choline-derived cellular activation has yet to be known; hence, this is a pioneer study looking at the emergence of new and novel molecular markers in the management and prevention of breast cancer. The exploitation of choline tracer strategy for staging and therapeutic response in breast cancer could promise a novel and potential role in breast cancer imaging.

1.3 Objectives

General Objective:

The aim of this study was to investigate the potential expression of choline as a molecular marker in signaling patients with breast cancer.

Specific Objectives were:

1. To assess the pharmacokinetic and dynamic of ^{18}F-FCH SUV_{max} uptake in detecting breast cancer.
2. To assess the ability of a dual tracer of ^{18}F-Fluorocholine (^{18}F-FCH) as compared to ^{18}F-Fluorodeoxyglucose(^{18}F-FDG) in detecting breast cancer.

3. To assess the ability of ^{18}F-FCH uptake values in detecting clinically aggressive histological phenotype in patients with receptor status of progesterone (PR), estrogen receptor(ER) and human epidermal growth factor receptor 2 (HER2) in breast cancer.
4. To evaluate the expression of CD47 and miRNA with the altered choline metabolism on ^{18}F-FCH PET/CT in breast cancer patients.

REFERENCES

Abdelbaky, A., Corsini, E., Figueroa, A.L., Fontanez, S., Subramanian, S., Ferencik, M., et al. Focal arterial inflammation precedes subsequent calcification in the same location: a longitudinal FDG-PET/CT study. *Circulation Cardiovascular Imaging*, 2013, 6(5), 747–754.

Ades, F., Zardavaz, D., Bozovic-Spasojevic, I., et al. Luminal B breast cancer:Molecular characterization,clinical management and future perceptive. *Journal Clinical Oncology* 2014, 32, 2794-803.

Aditya, D. PET-MRI: Challenges and new directions. *Indian Journal Nuclear Medicine* 2010, 25(1), 3–5.

Aboagye, E.O., Bhujwalla, Z.M. Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells. *Cancer Research* 1999, 59, 80–4.

Adelaide, J., Finetti, P., Bekhouche, I., Repellini, L., Geneix, J., Sircoulomb, F., Charafe-Jauffret, E., Cervera, N., Desplans, J., Parzy, D., Schoenmakers, E., Viens, P., Jacquemier, J., Birnbaum, D., Bertucci, F., Chaffanet, M. Integrated profiling of basal and luminal breast cancers. *Cancer Research 2007, 67, 11565-11575.*

Alexandrem, F., Pierre, V., Michael, W., Laurent, W., and Claire, R. A full nine-dimensional potential-energy surface for hydrogen molecule-water collisions. *Journal of Chemistery and Phyics* 2005;122:22-1102

Ali, S., Ahmad, A., Banerjee, S., Padhye, S., Dominiak, K., Schaffert, J.M., Wang, Z., Philip, P.A., Sarkar, F.H. Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF. *Cancer Research 2010, 70, 3606-3617.*

Aoyama, C., Liao, H., Ishidate, K. Structure and function of choline kinase isoforms in mammalian cells. *Progress in Lipid Research* 2004, 43, 266–281.

Asano, K., Nabeyama, A., Miyake, Y., et al. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. *Immunity* 2011, 34, 85–95.

Azmi, M.D. How to calculate your own sample size. www.drtamil.wordpress.com/2012/06/10/ fk6143-how-to-calculate-your-own-samplesize/.

Baccelli, I. Co-expression of MET and CD47 is a novel prognosticator for survival of luminal breast cancer patients. Oncotarget, 2014, 5, 8147–8160.

Baccelli, I. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nature Biotechnology 2013, 31, 539–544

Baenke, F., Peck, B., Miess, H., Schulze, A. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumor development. Disease Model Mechanism 2013, 6(6), 1353-63.

Baik, H.M., Su, M.Y., Yu, H., Nalcioglu, O., and Mehta, R. Quantification of choline-containing compounds in malignant breast tumors by H1 MR spectroscopy using water as an internal reference at 1.5 T, Magnetic Resonance Materials in Physics. Biology and Medicine 2006, 19(2), 96–104.

Balink, H. and Bruyn, G.A.W. The role of PET/CT in Cogan's syndrome. *Clinical of Rheumatology* 2007, 26(12), 2177–2179.

Brandt, K.R., Amrmi, K.K., Nelson, M.T., Everson, L.I., Emory, T.H., Tuttle, T.M., Yee, D., Garwood, M. Adding in vivo quantitative ^{1}H MR spectroscopy to improve diagnostic accuracy of breast MR imaging: preliminary results of Observer Performance Study at 4.0 T. Radiology 2005, 236, 465-475.

Barbagallo, B., Prescott, H.A., Boyle, P., Climer, J., Francis, M.M. A dominant mutation in a neuronal acetylcholine receptor subunit leads to motor neuron degeneration in Caenorhabditis elegans. *Journal of Neurosciences* 2010, 30, 13932–13942.

Bardou, V.-J., Arpino, G., Elledge, R.M., Osborne, C.K., Clark, G.M. Progesterone Receptor Status Significantly Improves Outcome Prediction Over Estrogen Receptor Status Alone for Adjuvant Endocrine Therapy in Two Large Breast Cancer Databases. *Journal of Clinical Oncology* 2003, 21(10), 1973–1979.

Bartella, L., Morris, E.A., Dershaw, D.D., Liberman, L., Thakur, S.B., Moskowitz, C., Guido, J., Huang, W. Proton MR spectroscopy with choline peak as malignancy marker improves positive predictive value for breast cancer diagnosis: preliminary study. *Radiology* 2006, 239, 686–692.

Basu, S., Chen, W., Tchou, J., et al. Comparison of triple-negative and estrogen receptor-positive/progesterone receptor-positive/HER2-negative breast carcinoma using quantitative fluorine-18 fluorodeoxyglucose/positron emission tomography imaging parameters: a potentially useful method for disease characterization. *Cancer* 2008, 112, 995–1000.

Behesthi, M., Haim, S., Zakavi, R., et al. Impact of 18F-choline PET/CT in prostate cancer patients with biochemical recurrence: influence of androgen deprivation therapy and correlation with PSA kinetics. *Journal of Nuclear Medicine* 2013, 54, 833–840.

Bing, Chen., Hongbin, Li., Xiao, Z., Pengbo, Y., Xinyu, L., Xia, Z., and Shufang, L. Roles of microRNA on cancer cell metabolism. *Journal of Translational Medicine*, 2012, 10-228.

Burstein, H.J. The distinctive nature of HER2-positive breast cancers. *The New England Journal of Medicine,* 2005,353 (16), 1652–1654.

Boellaard, R., Krak, N.C., Hoekstra, O.S., et al. Effects of Noise, Image Resolution, and ROI Definition on the Accuracy of Standard Uptake Values: A Simulation Study. *Journal Nuclear Medicine*, 2004, 45, 1519–1527.

Bolan, P.J., DelaBarre, L., Baker, E.H., Merkle, H., Everson, L.I., Yee, D., Garwood, M. Eliminating spurious lipid sidebands in 1H MRS of breast lesions. *Magnetic Resonance Medicine*, 2002, 48, 215–222.

Bolan, P.J., Baker, E.H., Pollema, M.G., Le, C.T., Kelcz, F., Lechner, M.C., Luikens, B.A., Carlson, R.A., Bong-Il, S., Chae, M.H., Hong, J.L., Sungmin, K., Shin, Y. L., Hae, W. K., et al. Prognostic value of primary tumor uptake on F-18 FDG PET/CT in patients with invasive ductal breast cancer. *Nuclear Medicine and Molecular Imaging 2011, 45,* 117–118.

Braun, L., Mietzsch, F., Seibold, P., Schneeweiss, A., Schirmacher, P., Chang-Claude,J., et al. Intrinsic breast cancer subtypes defined by estrogen receptor signalling-prognostic relevance of progesterone receptor loss. *Modern Pathology* 2013, 26(9), 1161–71.

Breast Cancer Estimated Incidence, Mortality and Prevalence Worldwide in 2012, *http:www.globocan.iarc.fr/factsheets/cancers/breast.asp*.

Brenner, W., Eary, J. F., Hwang, W., Vernon, C., & Conrad, E. U. Risk assessment in liposarcoma patients based on FDG PET imaging. *European Journal of Nuclear Medicine and Molecular Imaging* 2006, *33*(11), 1290–1295.

Cai-Xia, Z., Bin, L., Ying, L. & Lian, W. Folate intake and breast cancer prognosis: a meta-analysis of prospective observational studies. *European Journal of Cancer Prevention*, 2015, 24, 113–121.

Cancello, G., Maisonneuve, P., Rotmensz, N., Viale, G., Mastropasqua, M.G., Pruneri, G., et al. Progesterone receptor loss identifies Luminal B breast cancer subgroups at higher risk of relapse. *Annals of Oncology* 2013, 24(3), 661–668.

Candler, P.M., Hart, P.E., Barnett, M., Weil, R., Rees, J.H. A follow up study of patients with paraneoplastic neurological disease in the United Kingdom. *Journal of Neurology Neurosurgeon Psychiatry*, 2004, 75(10), 1411–5.

Cao, M.D., Dopkens, M., Krishnamachary, B., Vesuna, F., Gadiya, M.M., Lonning, P.E., Bhujwalla, Z.M., Gribbestad, I.S., Glunde, K. Glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) expression correlates with malignant choline phospholipid metabolite profiles in human breast cancer. *Nuclear Magnetic Resonance in Biomedicine* 2012, 25, 1033-1042.

Carey, K.A., Rebecca, J., Jennifer, L., Marianne, P., and Archie B. Breast Cancer Before Age 40 Years. *Seminars Oncology*, 2009, 36(3), 237–249.

Cecil, K.M., Schnall, Siegelman, E.S., and Lenkinski, R.E. The evaluation of human breast lesions with magnetic resonance imaging and proton magnetic resonance spectroscopy. *Breast Cancer Research and Treatment* 2001, 68(1), 45–54.

Chan, K.S., Espinosa, I., Chao, M., et al. Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proceeding of National Academic Sciences* U S A 2009, 106, 14016–14021.

Chan, J.A., Krichevsky, A.M., Kosik, K.S. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Research 2005, 65, 6029–6033.

Chen, K.,Chen, X.Positron emission tomography imaging of cancer biology:current status and future prospects. Seminars in Oncology, 2011, 38, 70-86.

Chin, K., DeVries, S., Fridlyand, J., Spellman, P.T., Roydasgupta, R., Kuo, W.L., Lapuk, A., Neve, R.M., Qian, Z., Ryder, T., Chen, F., Feiler, H., Tokuyasu, T., Kingsley, C., Dairkee, S., Meng, Z., Chew, K., Pinkel, D., Jain, A., Ljung, B.M., Esserman, L., Albertson, D.G., Waldman, F.M., Gray, J.W. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell* 2006, 10, 529-541.

Christopher, C.R., Timothy, A., Steven, L., Stephen, F.S., Scott, T., Amit, B., et al. 18F-FDG PET scanning correlates with tissue markers of poor prognosis and predicts mortality for patients after liver resection for colorectal metastases. *Journal of Nuclear Medicine*, 2007, *48*(5), 771–775.

Chiuve, S.E., Giovannucci, E.L., Hankinson, S.E., Zeisel, S.H., Dougherty, L.W., Willett, W.C., and Rimm, E.B. The association between betaine and choline intakes and the plasma concentrations of homocysteine in women. *The American Journal of Clinical Nutrition*, 2007, 86, 1073–1081.

Cho, E., Willett, W.C., Colditz, G.A., Fuchs, C.S., Wu, K., Chan, A.T., Zeisel, S.H., and Giovannucci, E.L. Dietary choline and betaine and the risk of distal colorectal adenoma in women. *Journal of National Cancer Institute* 2007, 99, 1224–1231.

Choi, M.G., Park, T.S., Carman, G.M. Phosphorylation of Saccharomyces cerevisiae CTP synthetase at Ser424 by protein kinases A and C regulates phosphatidylcholine synthesis by the CDP-choline pathway. *Journal of Biology and Chemistery*, 2003, 278, 23610–23616.

Christian, P. and Wolfgang, A. Weber. Tumor Cell Metabolism Imaging. *Journal Nuclear Medicine* 2008, 49, 43-63.

Cioffi, M., et al. Inhibition of CD47 effectively targets pancreatic cancer stem cells via dual mechanisms. *Clinical Cancer Research* 2015, 21(10), 2325–2337.

Coleman, R.E. Is quantitation necessary for oncological PET studies?. *European Journal of Nuclear Medicine Molecular Imaging* 2002, 29, 133–135.

Contractor, K., Challapalli, A., Barwick, T. Use of ^{11}C-choline PET-CT as a noninvasive method for detecting pelvic lymph node status from prostate cancer and relationship with choline kinase expression. *Clinical Cancer Research* 2011,17, 7673–7683.

Contractor, K.B., Kenny, L.M., Stebbing, J., et al. [11C]choline positron emission tomography in estrogen receptor-positive breast cancer. *Clinical Cancer Research* 2009, 15, 5503–5510.

Contractor, K., Kenny, L., Stebbing, J., Challapalli, A., Al-Nahhas, A., Palmieri, C., Shousha, S., Lewis, J., Hogben, K., De, Nguyen, Q., Coombes, R.C., Aboagye, E.O. Biological basis of [^{11}C] choline-positron emission tomography in patients with breast cancer: comparison with [11 F] fluorothymidine positron emission tomography. *Nuclear Medicine Communications* 2011, 32, 997-1004.

Corrigan, A.J., Schleyer, P.J., Cook, G.J. Pitfalls and artifacts in the use of PET/CT in oncology imaging. *Seminar Nuclear Medicine* 2015, 45 (6), 481–499.

Cortez, M.A., Welsh, J.W., Calin, G.A. Circulating microRNAs as noninvasive biomarkers in breast cancer. *Recent Results Cancer Research* 2012, 195, 151–161.

Coulouarn, C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS: Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene. 2009, 28: 3526-3536.

Damita, T., Marc, C., John, L. and Sandi, K. Pilot study of 18F-fluorocholine uptake in normal and malignant breast tissue. *Journal nuclear medicine* 2011,(1), 52-249.

DeGrado, T.R., Coleman, R.E., Wang, S., Baldwin, S.W., Orr, M.D., Robertson, C.N., Polascik, T.J., Price, D.T. *Synthesis and evaluation of 18F-labeled choline as an oncologic tracer for positron emission tomography: initial findings in prostate cancer. Cancer Research*. 2001, 61(1), 110–7.

DeGrado, T.R., Baldwin, S.W., Wang, S., Orr, M.D., Liao, R.P., Friedman, H.S., Reiman, R., Price, D.T., Coleman, R.E. *Synthesis and evaluation of (18)F-labeled choline analogs as oncologic PET tracers. Journal of Nuclear Medicine* 2001, 42(12), 1805–14

Deutsches Elektronen-Synchrotron, Medical Applications, A Research Centre of the Helmholtz Association, 2010.

De Molina, A.R., Banez-Coronel, R., Gutierrez, et al. Choline Kinase activation is a critical requirement for the proliferation of primary human mammary epithelial cells and breast tumor progression. *Cancer Research*, 2014, 18, 6732-6739.

Dorrius, M.D., De Vries, E.F., Slart, R.H., et al. Breast cancer: a new imaging approach as an addition to existing guidelines. *European Journal of Nuclear Medicine Molecular Imaging*, 2015, 42, 813–817.

Dunnwald, L.K.,Doot, R.K., Specht, J.M. PET tumor metabolism in locally advanced breast cancer patients undergoing neoadjuvant chemotherapy, value of static versus kinetic measures of fluorodeoxyglucose uptake. *Clinical Cancer Research*, 2011,17, 2400-2409.

Eberl, M.M., Fox, C.H., Edge, S.B., Carter, C.A., Mahoney, M.C. BI-RADS classification for management of abnormal mammograms. *Journal of American Board Family Medicine*. 2006, 19, 161–164.

Eliyahu, G., Kreizman, T., Degani, H. Phosphocholine as a biomarker of breast cancer: molecular and biochemical studies. *International Journal of Cancer* 2007, 120, 1721–1730.

Fathinul Fikri, A.S., Dharmendran, R., Vikneswaran, P., Nordin, A.J. 18F-FDG PET/CT as a potential predictor of survival in patient with oesophageal cancer: a preliminary result. *Journal of Abdomen Imaging* 2015, 40(6), 1457-1464.

Fathinul Fikri, A.S., Kroiss, A., Ahmad, A.Z., Zanariah, H., Lau, W.F., Uprimny, C., Donnemiller, E., Kendler, D., Nordin, A.J., Virgolini, I.J. Localization and prediction of malignant potential in recurrent pheochromocytoma/paraganglioma (PCC/PGL) using 18F-FDG PET/CT. *Acta Radiologica*, 2014, 55(5), 631-40.

Fathinul Fikri, A.S., Nordin, A.J., Eddie lau, E.F. 18[F] FDG-PET/CT is a useful molecular marker in evaluating tumour aggressiveness; a revised understanding of an in- vivo FDGPET imaging that alludes the alteration of cancer biology. *Cell Biochemistry and Biophysic* ,2014, 55 (5), 631-640.

Fathinul, F., Nordin, A.J., Eddie lau, E.F. 18[F]FDG-PET/CT is a Useful Molecular Marker in Evaluating Tumour Aggressiveness: A Revised Understanding of an In-Vivo FDG-PET Imaging that Alludes the Alteration of Cancer Biology. *Cell Biochemistry and Biophysics*, 2013, 66, 37-43.

Fathinul, F., Nordin, A. J., Zanariah, H., Kroiss, A., Uprimny, C., Donnemiller, E., Kendler, D., Virgolini, I.J. Localisation and prediction of recurrent phaechromocytoma/paraganglioma (PCC/PGL) using diagnostic ^{18}[F]FDG-PET/CT. *Cancer Imaging 2011, 3*(11), 114–115.

Fathinul Fikri, A.S., Razinul Aiman, F.F., Malini, K., Shazreen, S., Shahrun Niza, S. 18F FCH PET-CT and 18F FDG-PET in the evaluation of breast carcinoma: a pilot study. 18th Congress of the ASEAN Association Of Radiology (AAR 2017).Malaysia

Fei, X., Qi, M., Wu, B., Song, Y., Wang, Y., Li, T. MicroRNA-195-5p suppresses glucose uptake and proliferation of human bladder cancer T24 cells by regulating GLUT3 expression. Federations of European Biochemical Societies Letters 2012, 586, 392-397.

Feng, M., Chen, J.Y., Weissman-Tsukamoto, R., Volkmer, J.P., Ho, P.Y., McKenna, K.M. et al. Macrophages eat cancer cells using their own calreticulin as a guide: roles of TLR and Btk. *Proceedings of the National Academy of Sciences* USA 2015, 112, 2145–2150.

Ferdinando, C., Agostino, C., Carmelo, C., Vincenzo, G., Domenico, G., Federico, R., Gianpasquale, G., Giuseppe, L.C., Orazio, S. PET/CT with 18F–choline: Physiological whole bio-distribution in male and female subjects and diagnostic pitfalls on 1000 prostate cancer patients:18F–choline PET/CT bio-distribution and pitfalls. A southern Italian experience. *Nuclear Medicine and Biology* 2017, (51), 40–54.

Ferlay, J. *GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]*. Lyon, Fr. Int. Agency Res. Cancer. 2013. Available from: http://globocan.iarc.fr, accessed on 11/20/2014.

Fowler, J.S., Ido, T. Initial and subsequent approach for the synthesis of 18FDG.*Seminar in Nuclear Medicine* 2002, 32(1), 6-12.

Fulci, V., Chiaretti, S., Goldoni, M., Azzalin, G., Carucci, N., Tavolaro, S., Castellano, L., Magrelli, A., Citarella, F., Messina, M., et al. Quantitative technologies establish a novel microRNA profile of chronic lymphocytic leukemia. *Blood* 2007, 109, 4944–4951.

Garami, Z.,Hascsi, J.,Varga, T., Dinya, M., Tanyi, I., Garai, et al. The value of 18F-FDG PET/CT in early-stage breast cancer compared to traditional diagnostic modalities with an emphasis on changes in disease stage designation and treatment plan. *European Journal of Surgical Oncology* 2012, (1), 31-37.

Gillies, R.J., Gatenby, R.A. Adaptive landscapes and emergent phenotypes: Why do cancers have high glycolysis? *Journal of Bioenergetics Biomembranes* 2007, 39, 251–257.

Ginestier, C., Charafe-Jauffret, E., Penault-Llorca, F., Geneix, J., Adélaïde, J., Chaffanet, M., Mozziconacci, M.J., Hassoun, J., Viens, P., Birnbaum, D., Jacquemier, J. Comparative multi-methodological measurement of ERBB2 status in breast cancer. *Journal of Pathology* 2004, 202, 286-298.

Giorgio, T., Elisabetta, G., Davide, D.F., Maria, L.C., Vittoria, R., Maria, P., Alessandro, G. The role of positron emission tomography using carbon-11 and fluorine-18 choline in tumors other than prostate cancer:a systemic review. *Annals of Nuclear Medicine* 2012, 26, 451-461.

Giordano, S.H., Temin, S., Kirshner, J.J., et al. Systemic therapy for patients with advanced human epidermal growth factor receptor 2-positive breast cancer:American Society of Clinical Oncology clinical practice guideline. *Journal of Clinical Oncology* 2014, 32, 2078-2099.

Glunde, K., Bhujwalla, Z.M., Ronen, S.M. Choline metabolism in malignant transformation. *Nature Review of Cancer* 2011, 11, 835-848.

Glunde, K., Jie, C., Bhujwalla, Z. Molecular causes of the aberrant choline phospholipid metabolism in breast cancer. *Cancer Research* 2004, 64, 4270–42706.

Graveel, C.R., Calderone, H.M., Westerhuis, J.J., Winn, M.E. & Sempere, L.F. Critical analysis of the potential for microRNA biomarkers in breast cancer management. *Breast Cancer (Dove Medicine Press)* 2015, 7, 59–79.

Groheux, D., Moretti, J.L., Baillet, G. et al. Effect of (18)F-FDG PET/CT imaging in patients with clinical stage II and III breast cancer. *International Journal of Radiation Oncology Biology Physics* 2008, 71, 695–704.

Groheux, D, Espié, M., Giacchetti, S., Hindié, E. Performance of FDG PET/CT in the clinical management of breast cancer. *Radiology* 2013, 266(2), 388-405.

Groheux, D., Giacchetti, S., Moretti, J.L., et al. Correlation of high (18)F-FDG uptake to clinical, pathological and biological prognostic factors in breast cancer. *European Journal of Nuclear Medicine of Molecular Imaging* 2011,38, 426–435

Hazeman, Z., Fathinul, F., Shazreen, S., Shahrun Niza, S. Utility of 18 F- Flurocholine (FCH) PET -CT as predictor for breast cancer aggressiveness : An initial result.18th Congress of the ASEAN Association Of Radiology (AAR 2017).Malaysia

Hara, T., Kosako, N., Shinoura, N., Kondo, T. PET imaging of brain tumor with [methyl-^{11}C]choline. *Journal of Nuclear Medicine* 1997, 38, 842-847.

Haubrich, D.R., Wang, P.F., Wedeking, P.W. Distribution and metabolism of intravenously administered choline[methyl-^{3}H] and synthesis in vivo of acetylcholine in various tissues of guinea pigs. *Journal of Pharmacology and Experimental Therapeutic* 1975, 193, 246-255.

Higgs, G., Slack, F. The multiple roles of microRNA-155 in oncogenesis. *Journal of Clinical and Bioinformatics* 2013, 3(1), 17-23.

Hodgson, N.C., Gulenchyn, K.Y. Is there a role for positron emission tomography in breast cancer staging? *Journal of Clincal Oncology* 2008, 26, 712–720.

Holbro, T., Civenni, G., Hynes, N.E. The ErbB receptors and their role in cancer progression. *Experimental Cell Research* 2003, 284(1), 99–110.

Huang, W., Fisher, P.R., Dulaimy, K., Tudorica, L.A., O'Hea, B., Button, T.M. Detection of breast malignancy: diagnostic MR protocol for improved specificity. *Radiology* 2004, 232, 585–591.

Huang, R.S., Hu, G.Q., Lin, B., Lin, Z.Y., Sun, C.C. MicroRNA-155 silencing enhances inflammatory response and lipid uptake in oxidized low-density lipoprotein-stimulated human THP-1 macrophages. *Journal Investigations of Medicine* 2010, 58, 961–967.

Huang, Z., Zuo, C., Guan, Y., Zhang, Z., Liu, P., Xue, F.,et al. Misdiagnoses of 11C-Choline combined with 18F-FDG PET imaging in brains tumors. *Nuclear Medicine Communications* 2008, 29, 354-8.

Huimin, Z., Haiquan, L.u., Lisha, X., John, W., Bullen, Chuanzhao, Z., Debangshu S., Daniele, M., Gilkes, J.H., and Gregg, L. HIF-1 regulates CD47 expression in breast cancer cells to promote evasion of phagocytosis and maintenance of cancer stem cells. *Proceeding National Academy of Sciences U S A.* 2015, 112(45), 6215–6223.

Hynes, N.E., Lane, H.A. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nature Review Cancer* 2005, 5, 341–354.

Ikenaga, N., Otomo, N., Toyofuku, A., Ueda, Y., Toyoda, K., Hayashi, T., Nishikawa, K., Tanaka, M. Standardized uptake values for breast carcinomas assessed by fluorodeoxyglucose-positron emission tomography correlate with prognostic factors. *Surgery centers Management* 2007, 73(11), 1151-1157.

Iorio, M.V., Ferracin, M., Liu, C.G., et al. MicroRNA gene expression deregulation in human breast cancer. *Cancer Reserach* 2005, 65(16),7065–7070.

Ivan, P., Ines, D., Maria, J., Josep, M. et al. PET tracers for clinical Imaging of Breast Cancer. *Journal of Oncology* 2012,9, 9-18.

Izquierdo-Garcia, D., Davies, J.R., Graves, M.J. Comparison of methods for magnetic resonance-guided[18-F]fluorodeoxyglucose positron emission tomography in human carotids arteries:reproducibility,partial volume correction,and correlation between methods. *Stroke* 2009, 40, 18-93.

Jacobs, M.A., Stearns, V., Wolff, A.C., Macura, K., Argani, P., Khouri, N., Tsangaris, T., Barker, P.B., Davidson, N.E., Bhujwalla, Z.M., et al. Multiparametric magnetic resonance imaging, spectroscopy and multinuclear (^{23}Na) imaging monitoring of preoperative chemotherapy for locally advanced breast cancer. *Academics Radiology* 2010, 17, 1477–1485.

Jadvar, H. Molecular imaging of prostate cancer with 18F-fluorodeoxyglucose PET. *Nature Review Urology* 2009, 6, 317-323.

Jaiswal, S., Chao, M.P., Majeti, R. and Weissman, I.L. Macrophages as mediators of tumor immunosurveillance. *Trends Immunology* 2010, 31, 212–219.

James, H.F.R, Jagat, N., William, S.H., Renu, V., Josef, M., Mike, K., Nobuhiro, T., Valentin, F., Elizabeth, A.W, Zahi, A.F, and Ahmed, A.T. Imaging Atherosclerotic Plaque Inflammation by Fluorodeoxyglucose With Positron

Emission Tomography: Ready for Prime Time? *Journal of Americans College Cardiology* 2010, 55, 2527-2535.

Jeong, Y.J., Kang, D.Y., Yoon, H.J., Son, H.J. Additional value of F-18 FDG PET/CT for initial staging in breast cancer with clinically negative axillary nodes. *Breast Cancer Research Treatments* 2014, 145, 137–142.

Jingwei, C., James, A.D.C., Michele, M.F., and Brian S.S. Cellular Trasformation by Simian Virus 40 and murine Polyoma Virus T antigens. *Seminar Cancer Biology* 2009, 19(4),218-228.

Junker, A., Krumbholz, M., Eisele, S., et al: MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. *Brain* 2009, 132, 3342–3352.

Katz-Brull, R., Lavin, P.T., and Lenkinski, R.E. Clinical utility of proton magnetic resonance spectroscopy in characterizing breast lesions. *Journal of the National Cancer Institute*, 2002, 94(16), 1197–1203.

Katz-Brull, R., Seger, D., Rivenson-Segal, D., Rushkin, E., Degani, H. Metabolic markers of breast cancer: enhanced choline metabolism and reduced choline-ether phospholipid synthesis. Cancer Research 2002, 62, 1966-1970.

Kelloff, G.J., Hoffman, J.M., Johnson, B., et al. Progress and promise of FDG-PET imaging for cancer patient management and oncologic drug development. *Clinical Cancer Research* 2005, 11, 2785–808.

Kenny, L.M., Vigushin, D.M., Al-Nahhas, A., et al .Quantification of cellular proliferation in tumor and normal tissues of patients with breast cancer by [18F]fluorothymidine-positron emission tomography imaging: evaluation of analytical methods. *Cancer Research* 2005, 65, 10104–10112.

Kent, C. Regulatory enzymes of phosphatidylcholine biosynthesis: a personal perspective. *Biochimica et Biophysica Acta* 2005, 1733, 53–66.

Kida, K., Nakajima, M., Mohri, T., Oda, Y., Takagi, S., Fukami, T., Yokoi, T. PPARalpha is regulated by miR-21 and miR-27b in human liver. *Pharmaceutical Research* 2011, 28, 2467–2476.

Kuhl, C.K. and Schild, H.H. Dynamic image interpretation of MRI of the breast. *Journal of Magnetic Resonance Imaging*, 2000, 12(6), 965–974.

Kuhajda, F.P. Fatty acid synthase and cancer: new application of an old pathway. *Cancer Research* 2006, 66, 5977–5980.

Kwee, S.A., DeGrado, T.R., Talbot, J.N., et al. Cancer imaging with fluorine-18-labeled choline derivatives. *Seminars in Nuclear Medicine* 2007, 37, 420–428.

Kwee, S.A., Coel, M.N., Lim, J. et al. Prostate cancer localization with 18fluorine fluorocholine positron emission tomography. *Journal of Urology* 2005, 173, 252-255.

Lee, J.E., Giovannucci, E., Fuchs, C.S., Willett, W.C., Zeisel, S.H., Cho, E. Choline and betaine intake and the risk of colorectal cancer in men. *Cancer Epidemiology, Biomarkers & Prevention* 2010, 19, 884–887.

Lee, T.K. Blockade of CD47-mediated cathepsin S/protease-activated receptor 2 signaling provides a therapeutic target for hepatocellular carcinoma. *Hepatology* 2014, 60(1), 179–191.

Letessier, A., Sircoulomb, F., Ginestier, C., Cervera, N., Monville, F., Gelsi-Boyer, V., Esterni, B., Geneix, J., Finetti, P., Zemmour, C., Viens, P., Charafe-Jauffret, E., Jacquemier, J., Birnbaum, D., Chaffanet, M. Frequency, prognosis impact and subtype association of 8p12, 8q24, 11q13, 12p13, 17q12 and 20q13 amplifications in breast cancers. BMC Cancer 2006, 6, 245-255.

Li, S.X., Sjolund, A., Harris, L., Sweasy, J.B. DNA repair and personalized breast cancer therapy. *Environment Molecular Mutagen* 2010, 51, 897–908.

Li, Z., Vance, D. Phosphatidylcholine and choline homeostasis. *Journal of Lipid Research* 2008, 49, 1187–1194.

Lin, S. & Gregory, R. I. MicroRNA biogenesis pathways in cancer. *Nature Review Cancer* 2015, 15, 321–333.

Liu, X. CD47 blockade triggers T cell-mediated destruction of immunogenic tumors. *Nature Medicine* 2015, 21(10), 1209–1215.

Lowe, V.J., Hoffman, J.M., DeLong, D.M., et al. Semiquantitative and visual analysis of FDG-PET images in pulmonary abnormalities. *Journal of Nuclear Medicine* 1994, 35, 1771–1776.

Makoto, N., Koshi, M., Akemi, K., Hideshi, I., Fumiaki, T., Tsuyoshi, N., Takanobu S. Correlated Expression of CD47 and SIRPA in Bone Marrow and in Peripheral Blood Predicts Recurrence in Breast Cancer Patients. *Clinical cancer research* 2010, 1158(10), 1078-0432.

Mankoff, D.A., Eary, J.F., Link, J.M., Muzi, M., Rajendran, J.G., Spence, A.M., Krohn, K.A. Tumor-specific positron emission tomography imaging in patients: [18F] fluorodeoxyglucose and beyond. *Clinical Cancer Research* 2007,13,3460–3469.

Manna, P.P., Frazier, W.A. The mechanism of CD47-dependent killing of T cells: heterotrimeric Gi-dependent inhibition of protein kinase A. *Journal of Immunology* 2003, 170, 3544–53.

Marcelo, F. Di, C., Rory, H. New Technology for Noninvasive Evaluation of Coronary Artery Disease. *Circulation* 2007, 115, 1464-1480.

Majeti, R., Chao, M.P., Alizadeh, A.A., et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* 2009,138, 286–99.

Mar-Aguilar, F., Mendoza-Ramirez, J.A., Malagon-Santiago, I. et al .Serum circulating microRNA profiling for identification of potential breast cancer biomarkers. *Disease Markers* 2013, 34, 163–169.

Mark, P.S., Dunphy, D.O., Alvin, F., Steven, M., Larson, M.D. Association of Vascular ^{18}F-FDG Uptake with Vascular Calcification. *Journal of Nuclear Medicine* 2005, 46, 1278-1284.

Mark, P.M., Jonathan, H., John, R.C., Hedley, C.A.E. The utility of FDG PET/CT in clinically Suspected Paraneoplastic Neurological Syndrome: A literature Review and Retrospective Case Series 2017, 8, 238-244.

Maria, T., Grinde, Nirma, S., Siver, A., Moestue, Einar, A., Eldrid, B., Alexander, K., et al. Interplay of choline metabolites and genes in patient-derived breast cancer xenografts. *Breast Cancer Research* 2014, 16, 5-10.

Marjeti, R., Chao, M.P., Alizadeh, A.A. Cd47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *NCBI Journal* 2009, 138, 286-299.

Matozaki, T., Murata, Y., Okazawa, H., Ohnishi, H. Functions and molecular mechanisms of the CD47-SIRPα signalling pathway. *Trends Cell Biology* 2009, 19, 72–80.

Mattiske, S., Suetani, R.J., Neilsen, P.M., et al. The oncogenic role of miR-155 in breast cancer. *Cancer Epidemiology Biomarkers Previous* 2012, 21(8), 1236–1243.

Meisamy, S., Bolan, P.J., Baker, E. H., et al. Neoadjuvant chemotherapy of locally advanced breast cancer: predicting response with in vivo 1H MR spectroscopy—a pilot study at 4 T. *Radiology* 2004, 233(2), 424–431.

Menendez, J.A., Lupu, R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nature Review Cancer* 2007, 7, 763–777.

Menendez, J.A., Vellon, L., Mehmi, I., et al. Inhibition of fatty acid synthase (FAS) suppresses HER2/neu (erbB-2) oncogene overexpression in cancer cells. *Proceeding of National Academy Sciences* USA 2004, 101, 10715–10720.

Menezes, L.J., Kotze, C.W., Hutton, B.F., Endozo, R., Dickson, J.C., Cullum, I., et al. Vascular inflammation imaging with 18F-FDG PET/CT: when to image? *Journal of Nuclear Medicine* 2009, 50(6), 854–857.

Mertens, K., Slaets, D., Lambert, B., Acou, M., De Vos, F., Goethals, I. PET with (18)F-labelled choline-based tracers for tumor imaging: a review of the literature.

European Journal of Nuclear Medicine Molecular Imaging 2010, 37, 2188-2193.

Mimori, K., Fukagawa, T., Kosaka, Y., et al. Hematogenous metastasis in gastric cancer requires isolated tumor cells and expression of vascular endothelial growth factor receptor-1. *Clinical Cancer Research* 2008, 14, 2609–2616.

Molina, R., Banez-Coronel, M., Gutierrez, R. Choline kinase activation is a critical requirement for the proliferation of primary human mammary epithelial cells and breast tumor progression. *Cancer Research*, 2004, 64(18), 6732–6739.

Moody, S.E., Sarkisian, C.J., Hahn, K.T. Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell* 2002, 2(6), 451–461.

Mulrane, L., McGee, S.F., Gallagher, W.M., & O'Connor, D. miRNA dysregulation in breast cancer. *Cancer Research* 2013, 73, 6554–6562

Narayanan, D., Madsen, K.S., Kalinyak, J.E., and Berg, W.A. Interpretation of positron emission mammography and MRI by experienced breast imaging radiologists:performance and observer reproducibility. *American Journal of Roentgenology*, 2011, 4, 971-981.

Ng, E.K., Chong, W.W., Jin, H., et al. Differential expression of microRNAs in plasma of colorectal cancer patients: A potential marker for colorectal cancer screening. *Gut* 2009,58, 1375−1381.

Normand, P., and Delphine, D. Molecular Imaging of metabolism in cancer metastasis. *Cancer Forum* 2014, 38(2), 124-128.

Onitilo, A.A., Engel, J.M., Greenlee, R.T., Mukesh, B.N. Breast cancer subtypes based on ER/PR and her-2 overexpression:comparison of clinicophatolohic features and survival. *Clinical Medicine Research* ,2009, 7, 4-13.

Ora, I. and Dominique, D. Normal distribution, Variants, Pitfalls and artifacts. Springer New York 2010, 1, 35-96.

Olsson, M., Oldenborg, P.A. CD47 on experimentally senescent murine RBCs inhibits phagocytosis following Fcγ receptor-mediated but not scavenger receptor-mediated recognition by macrophages. *Blood* 2008, 112, 4259–4267.

Oude Munnink, T.H., Nagengast, W.B., Brouwers, A.H., Schröder, C.P., Hospers, G.A., et al. Molecular imaging of breast cancer. *Breast* 2009, 18(3), 66-73.

Onodera, Y., Nam, J.M., Bissell, M.J. Increased sugar uptake promotes oncogenesis via EPAC/RAP1 and O-GlcNAc pathways. *Journal Clinical Investigation* 2014, 124(1), 367-384.

Paola, A., Betancur, Brian, J., Abraham, Ying, Y., Yiu, et al. A CD47-associated super-enhancer links pro-inflammatory signalling to CD47 upregulation in breast cancer. *Nature communication* 2017, 10, 1038-14802.

Parise, C.A., Bauer, K.R., Brown, M.M., Caggiano, V. Breast cancer subtypes as defined by the estrogen receptor (ER), progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2) among women with invasive breast cancer in California, 1999-2004. *Breast* 2009, 15, 593–602.

Paulmier, B., Duet, M., Khayat, R., et al. Arterial wall uptake of fluorodeoxyglucose on PET imaging in stable cancer disease patients indicates higher risk for cardiovascular events. *Journal of Nuclear Cardiology* 2008, 15, 209–217.

Penry, J., Manore, M. Choline: an important micronutirent for maximal endurance-exercise performance? *International Journal Sport Nutritions Exercise Metabolism* 2008, 18, 191–203.

Picchio, M.C., Messa, C., Landoni, et al. Value of [11C]choline-positron emission tomography for re-staging prostate cancer: a comparison with [18F]fluorodeoxyglucose-positron emission tomography. *Journal of Urology* 2003, 169, 1337-1340.

Precht, L.M., Lowe, K.A., Atwood, M., Beatty, J.D. Neoadjuvant chemotherapy of breast cancer: tumor markers as predictors of pathologic response, recurrence, and survival. *Breast* 2010, 16, 362–368.

Punglia, R.S., Kuntz, K.M., Winer, E.P., Weeks, J.C., Burstein, H.J. The impact of tumor progesterone receptor status on optimal adjuvant endocrine therapy for postmenopausal patients with early-stage breast cancer. *Cancer* 2006, 106(12), 2576–2582

Rakha, E.A., Ellis, I.O. Triple-negative/basal-like breast cancer: review. *Pathology* 2009, 41, 40–47\

Ramirez, de Molina, A., Rodriguez-Gonzalez, A., Gutierrez, R., et al. Overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate, and colorectal human cancers. *Biochemical and Biophysical Research Communication* 2002, 296, 580–3.

Ramirez, de Molina A., Gallego-Ortega, D., Sarmentero-Estrada, J., et al. Choline kinase as a link connecting phospholipid metabolism and cell cycle regulation: implications in cancer therapy. *The International Journal of Biochemistry and Cell Biology* 2008, 40, 1753–63.

Ramirez, de Molina, A., Sarmentero-Estrada, J., Belda-Iniesta, C., et al. Expression of choline kinase α to predict outcome in patients with early-stage non-small-cell lung cancer: a retrospective study. *Lancet Oncology* 2007, 8, 889–97.

Reeps, C., Bundschuh, R.A., Pellisek, J., Herz, M., Van Marwick, S., Schwaiger, M., et al. Quantitative assessment of glucose metabolism in the vessel wall of abdominal aortic aneurysms: correlation with histology and role of partial volume correction. *International Journal Cardiovascular Imaging* 2013, 29(2), 505–512.

Reimer, S.E., Adler, L.P., & Bloom, A.D. Prospective evaluation of PET FDG in FNA indeterminate thyroid nodules (abstract). *Journal of Nuclear Medicine*, 1998, *39*, 119-123.

Rhodes, A., Jasani, B. The oestrogen receptor-negative/progesterone receptor-positive breast tumour: a biological entity or a technical artefact?. *Journal of Clinical Pathology* 2009, 62(1), 95–106.

Rice, A., Quinn, C.M. Angiogenesis, thrombospondin, and ductal carcinoma in situ of the breast. *Journal of Clinical Pathology* 2002, 55, 569–574.

Roivainen, A., Forsback, S., Groenroos, T., et al. Blood metabolism of [methyl-^{11}C] choline:implications for in vivo imaging with positron emission tomography. *European Journal of Nuclear Medicine* 2000, 27, 25-32.

Rominger, A., Saam, T., Wolpers, S., et al .18F-FDG PET/CT identifies patients at risk for future vascular events in an otherwise asymptomatic cohort with neoplastic disease. *Journal of Nuclear Medicine* 2009, 50(10), 1611–1620.

Rosen, E.L., Eubank, W.B., Mankoff, D.A. FDG PET, PET/CT, and breast cancer imaging. *Radiographics* 2007, 27 (1), 215–229.

Rottiers, V., Naar, A.M. MicroRNAs in metabolism and metabolic disorders. *Nature Review Molecular Cell Biology* 2012, 13, 239–250.

Roy, V., Perez, E.A. Beyond trastuzumab: small molecule tyrosine kinase inhibitors in HER-2-positive breast cancer. The Oncologist 2009, 14 (11), 1061–1069.

Sanghwa, K., Hyung, S.P., Jee, Y.K., Jegyu, R., Seho, P., and Seung, I.K. Comparisons of Oncologic Outcomes between Triple-Negative Breast Cancer (TNBC) and Non-TNBC among Patients Treated with Breast-Conserving Therapy. *Yonsei Medical Journal*, 2016, 57(5), 1192-1198.

Schaeffer, P., Mulcahy, H.E., Meyer, P., et al. Telomerase RNA as a detection marker in the serum of breast cancer patients. *Clinical Cancer Research* 2000, 6, 3823–3826.

Scheidhauer, K., Walter, C., Seemann, M.D. FDG PET and other imaging modalities in the primary diagnosis of suspicious breast lesions. *European journal Nuclear Medicine of Molecular Imaging* 2004, 31, 70-79.

Schwarzenbock, S., Souvatzoglou, M. and Krause, B.J. Choline PET and PET/CT in Primary Diagnosis and Staging of Prostate Cancer. *Theranostics*. 2012, 2(3), 318–330.

Serpico, D., Molino, L. & Cosimo, S.D. microRNAs in breast cancer development and treatment. *Cancer Treatment Review* 2014, 40, 595–604.

Shigemasa, S., Takehiko, Y., Narakitaka, T., Makoto, S., Akihiko, S., Takanori, I., et al. CD47 expression regulated by the miR-133a tumor suppressor is a novel prognostic marker in esophageal squamous cell carcinoma. *Cancers* 2012, 10, 465-472.

Shin, H.J., Baek, H.M., Cha, J.H., Kim, H.H. Evaluation of breast cancer using proton MR spectroscopy: total choline peak integral and signal-to-noise ratio as prognostic indicators. AJR *American Journal of Roentgenology* 2012, 198, 488–497. Sick, E., Jeanne, A., Schneider, C., Dedieu, S., Takeda, K., Martiny, L . CD47 update: a multifaceted actor in the tumour microenvironment of potential therapeutic interest. *British Journal of Pharmacology* 2012, 167 (7), 1415–1430.

Shiono, S. Positron emission tomography/computed tomography and lymphovascular invasion predict recurrence in stage I lung cancers. *Journal of Thoracic Oncology*, 2011, *6*, 43–47.

Si, H., Sun, X., Chen, Y. et al. Circulating microRNA-92a and microRNA-21 as novel minimally invasive biomarkers for primary breast cancer. *Journal Cancer Research Clinical Oncology* 2013, 139, 223–229.

Sitter, B., Lundgren, S., Bathen, T.F., Halgunset, J., Fjosne, H.E., Gribbestad, I.S. Comparison of HR MAS MR spectroscopic profiles of breast cancer tissue with clinical parameters. *NMR Biomedicine* 2006, 19, 30-40.

Sitter, B., Sonnewald, U., Spraul, M., Fjosne, H.E., Gribbestad, I.S. High-resolution magic angle spinning MRS of breast cancer tissue. NMR Biomedicine 2002, 15, 327-337.

Soerjomataram, I., Louwman, M.W., Ribot, J.G., Roukema, J.A., Coebergh, J.W. An overview of prognostic factors for long-term survivors of breast cancer. *Breast Cancer Research Treatment* 2008, 107, 309–330.

Soo, Y.B., Sangmin, K., Jun, H.L., Hyun-chul, L., Se, K.L., Won, H.K., Seok, W.K., Jeong, E.L. and Seok, J.N. Poor prognosis of single hormone receptor- positive breast cancer: similar outcome as triple-negative breast cancer. *BMC cancer* 2015,15, 138-147.

Sotiriou, C., Piccart, M.J. Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care? *National Review Cancer* 2007, 7, 545–553.

Specht, J.M., Mankoff, D.A. Advances in molecular imaging for breast cancer detection and characterization. *Breast Cancer Research* 2012, 14, 200-206.

Sun, Y., Wang, M., Lin, G., et al. Serum microRNA-155 as a potential biomarker to track disease in breast cancer. *PLoS One* 2012, 7(10), 47003-47010.

Stead, L., Brosnan, J., Brosnan, M., Vance, D., Jacobs, R. Is it time to reevaluate methyl balance in humans. *American Journal of Clinical Nutrition* 2006,83, 5–10.

Steinert, G. Immune escape and survival mechanisms in circulating tumor cells of colorectal cancer. *Cancer research* 2014, 74, 1694-1704.

Sutinen, E., Nurmi, M., Roivainen, A., et al. Kinetics of [(11)C] choline uptale in prostate cancer: a PET study. *European Journal Nuclear Medicine of Molecular Imaging* 2004, 31, 317-324.

Sven,K., Branko, D., Ksenija, M., Damir, V., and Antonije J. Distribution of Ki-67 values within Her2 and ER/PgR expression variants of ductal breast cancers as a potential link between IHC features and breast cancer biology. *BMC cancer* 2017, 17, 231-244

Takahashi, R.U., Miyuzaki, H. and Ochiya, T. The roles of microRNAs in breast cancer. *Cancers* 2015, 7,598-616.

Talbot, J.N., Gutman, F., Fartoux, L., Grange, J.D., Ganne, N., Kerrou, K., Grahek, D., Montravers, F., Poupon, R., Rosmorduc, O. PET/CT in patients with hepatocellular carcinoma using [^{18}F]fluorocholine: Preliminary comparison with [^{18}F]FDG PET/CT. *European Journal of Nuclear Medicine Molecular Imaging* 2006, 33(11), 1285-1290.

Talbot, J.N., Fartoux, L., Balogova, S., Nataf, V., Kerrou, K., Gutman, F., et al. Detection of hepatocellular carcinoma with PET/CT:a prospective comparison of 18F-fluorocholine and 18F-FDG in patients with cirrhosis or chronic liver disease. Journal of Nuclear Medicine 2010, 51, 1699-706.

Tan, H., Chen, L., Guan, Y., Lin, X. Comparison of MRI,F-18 FDG,and 11C-choline PET/CT for their potentials in differentiating brain tumor recurrence from brain tumor necrosis following radiotherapy. *Clinical Nuclear Medicine* 2011, 36, 978-81.

Tariq, S., Flonne, W., Marie-France, P., Paul, T.W. et al.,.Choline kinase overexpression increases invasiveness and drug resistance of human breast cancer cells. *NMR Biomedicine* 2010, 23(6), 633–642.

Testa, C.R., Schiavina, R. Lodi, et al. Prostate cancer: sextant localization with MR imaging, MR spectroscopy, and 11C-choline PET/CT. *Radiology*, 2007, 244(3), 797-806.

Thie, J.A. Understanding the standardized uptake value, its methods, and implications for usage. *Journal of Nuclear Medicine* 2004, 45, 1431–1434.

Timothy, R., DeGrado, Robert, E., David, T.P., Shuyan, W.R. and Edward, C. Pharmocokinetic and Radiation Dosimetry of 18F-fluorocholine. *Journal of Nuclear Medicine* 2002, 43, 92-96.

Tindall, E., Monare, R., Petersen, D.C. et al. Clinical Presentation of Prostate Cancer in Black South Africans. *The Prostate* 2014, 74, 880-891.

Tolvanen, T., Yli-Kerttula, T., Ujula, T., Autio, A., Lehikoinen, P., Minn, J., Roivinen, A. Biodistribution and radiation dosimetry of[11C] choline: a comparison between rat and human data. *European Journal of Nuclear Medicine Molecular Imaging* 2010, 37, 874-83.

Tozaki, M., Sakamoto, M., Oyama, Y., Maruyama, K., Fukuma, E. Predicting pathological response to neoadjuvant chemotherapy in breast cancer with quantitative 1H MR spectroscopy using the external standard method. *Journal of Magnetic Resonance Imaging* 2010, 31, 895–902.

Townsend, D.W. Dual-modality imaging: combining anatomy and function. *Journal of Nuclear Medicine* 2008, 49, 938-955.

Van de Sande, T., De Schrijver, E., Heyns, W., Verhoeven, G., Swinnen, J.V. Role of the phosphatidylinositol 3′-kinase/PTEN/Akt kinase pathway in the overexpression of fatty acid synthase in LNCaP prostate cancer cells. *Cancer Research* 2002, 62, 642–646.

Van Schooneveld, E., Wildiers, H., Vergote, I., Vermeulen, P.B., Dirix, L. Y. & Van Laere, S. J. Dysregulation of microRNAs in breast cancer and their potential role as prognostic and predictive biomarkers in patient management. *Breast Cancer Research* 2015, 17, 19-21.

Vici, P., Pizzuti, L., Natoli, C.,et al. Triple positive breast cancer:A distinct subtype?*Cancer Treatments Review* ,2015, 41, 69-76.

Wang, X., Koch, S. Positron emission tomography/computed tomography potential pitfalls and artifacts. *Current Problem in Diagnostic Radiology* 2009, 38, 156–169.

Ward, P.S., Thompson, C.B. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer cell* 2012, 21(3), 297-308.

Wehrl, H.F., Schwab, J., Hasenbach, K., et al. Multimodal elucidation of choline metabolism in a murine glioma model using magnetic resonance spectroscopy and ^{11}C-choline positron emission tomography. *Cancer Research* 2013, 73(5), 1470–1480.

Westbrook, C., Roth, C.K., Talbot, J. 4th edition. John Wiley & Sons, Inc.; London: 2011. MRI in Practice

Wolff, A.C., Hammond, M.E., Schwartz, J.N., Hagerty, K.L., Allred, D.C., Cote, R.J., Dowsett, M., Fitzgibbons, P.L., Hanna, W.M., Langer, A., McShane, L.M., Paik, S., et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Journal of Clinical Oncology* 2007, 25(1), 118-145.

Wu, X., Somlo, G., Yu, Y. De novo sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. *Journal of Translational Medicine* 2012, 10, 42-49.

Xu, X., Gammon, M., Zeisel, S., et al. Choline metabolism and risk of breast cancer in a population-based study. *FASEB Journal* 2008,22, 1–8.

Xu, X., Gammon, M.D., Zeisel, S.H., Bradshaw, P.T., Wetmur, J.G., Teitelbaum, S.L., Neugut, A.I., Santella, R.M., Chen, J. High intakes of choline and betaine reduce breast cancer mortality in a population-based study. *FASEB J* 2009, 23(11), 4022-4028.

Yanaihara, N., Caplen, N., Bowman, E., Seike, M., Kumamoto, K., Yi, M., Stephens, R.M., Okamoto, A., Yokota, J., Tanaka, T., et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006, 9, 189–198.

Yin, W., Jiang, Y., Shen, Z., Shao, Z., Lu, J. Trastuzumab in the adjuvant treatment of HER-2 positive early breast cancer patients;a meta-analysis of published randomized controlled trials. *PLoS One* 2011, 6, 21030-21039.

Yon, M.S., Kyung, S.L., Byung-Tae, K., Joon, Y.C., Young, M.S., & Chin, A.Y. 18F-FDG PET/CT of thymic epithelial tumors: Usefulness for distinguishing and staging tumor subgroups. *Journal of Nuclear Medicine*, 2006, *47*, 1628–1629.

Zhang, C.X., Pan, M.X., Li, B., Wang, L., Mo, X.F., Chen, Y.M., Lin, F.Y., Ho, S.C. Choline and betaine intake is inversely associated with breast cancer risk: a two-stage case-control study in China. *Cancer Sciences* 2013, 104(2), 250-8.

Zainal, A.O., Nor Saleha, I.T. (2011), National Cancer Registry Report Malaysia Cancer Statistics – Data and Figure 2007,Malaysia, National Cancer Registry Ministry of Health, Malaysia.

Zeisel, S. Choline: Critical role during fetal development and dietary requirements in adults. *Annual Review of Nutritions* 2006, 26, 229–250.

Zeisel, S., Mar, M.H., Howe, J., Holden, J. Concentrations of choline-containing compounds and betaine in common foods. *Journal of Nutritions* 2003, 133, 1302–1307.

Zhang, C.M., Zhao, J., Deng, H.Y. MiR-155 promotes proliferation of human breast cancer MCF-7 cells through targeting tumor protein 53-induced nuclear protein 1. *Journal of Biomedical Sciences* 2013, 20, 79-89.

Zhang, H. et al. *HIF-1 regulates CD47 expression in breast cancer cells to promote evasion of phagocytosis and maintenance of cancer stem cells*. *Proceedings of National Academy Sciences* USA 2015, 112, 6215–6223.

Zhu, S., Wu, H., Wu, F., Nie, D., Sheng, S., Mo, Y.Y. MicroRNA-21 targets tumor suppressor genes in invasion and metastasis. *Cell Research* 2008, 18, 350–359.