THE VALUE OF DELAYED IMAGING IN DETECTION OF RECURRENT PROSTATE CANCER USING GALLIUM-68 PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA) POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY (PET/CT).

By

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DISCLAIMER

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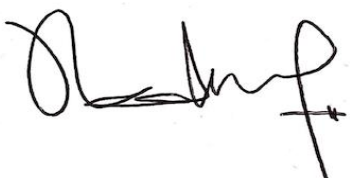

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ABBREVIATIONS

Ga-68	Gallium-68
Ga-68 PSMA	Gallium-68 Prostate Specific Membrane Antigen
PSA	Prostate specific antigen
DRE	Digital rectal examination
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
Tc-99m	Technetium-99m
F-18 FLT	Fluorine-18 Flurothymidine
mp-MRI	multiparametric magnetic resonance imaging
PI-RADS	Prostate Imaging Reporting and Data System
ISUP	International Society of Urological Pathology
AJCC	American Joint Committee on Cancer
TNM	Tumour-node-metastasis
ROI	Region of interest
SUV	Standardised Uptake Value
SUVmax	Standardised Uptake Value Maximum
RP	Radical Prostatectomy
PLND	Pelvic Lymph Node Dissection
EBRT	External Beam Radiotherapy
IMRT	Intensity Modulated Radiotherapy
GS	Gleason Score

SYMBOLS

kBq/ml	kilobecquerel per milliliter
g/ml	gram per milliliter
U/L	units per liter
MBq	megabecquerel

ABSTRAK

TUJUAN KAJIAN: Ujian darah yang dipanggil PSA atau antigen spesifik prostat dan kaedah pengimejan diagnostik berfungsi untuk mengesan kanser prostat selepas pesakit menjalani terapi awal prostat kanser samada melalui pembedahan prostatektomi radikal atau rawatan radioterapi. Penemuan radiotracer Ga-68 prostat spesifik membran antigen (PSMA) telah membolehkan penggunaan modaliti pengimejan Ga-68 PSMA PET/CT untuk mengesan peringkat dan gred kanser prostat di kalangan kanser prostat berulang yang boleh membawa perubahan dalam pilihan rawatan kanser. Walaubagaimanapun, kajian Ga-68 PSMA PET/CT mempunyai limitasi dalam membezakan samada tisu prostat adalah benigna atau malignan disebabkan oleh taburan fisiologi dan kesan daripada terapi di bahagian pelvis. Tujuan ini adalah untuk mengkaji nilai tambahan bagi pengimejan tertunda Ga-68 PSMA PET/CT bagi meningkatkan kadar pengesanan kanser prostat di kalangan pesakit prostat berulang.

METODOLOGI: Kajian retrospektif ini melibatkan seramai 87 pesakit yang telah didiagnos dengan kanser prostat berulang melalui peningkatan bacaan ujian PSA selepas rawatan pembedahan prostat atau radioterapi. Protokol pengimejan melibatkan ujian skan PET/CT seluruh badan pada dua tempoh masa iaitu 1 jam dan 3 jam selepas suntikan radiofarmaseutikal Ga-68 PSMA. Data klinikal dan pengimejan telah dianalisa melalui kaedah visual dan semikuantitatif melalui nilai Standardized Uptake Value (SUV).

KEPUTUSAN: Seramai 87 pesakit telah menjalani skan Ga-68 PSMA PET/CT. Kadar pengesanan lesi positif kanser prostat adalah sebanyak 84%. Jumlah keseluruhan lesi positif PSMA sebanyak 158 lesi telah dikenalpasti di dalam 73 pesakit. Semua lesi positif PSMA telah dikesan pada peringkat skan awal Ga-68 PSMA PET/CT pada tempoh 1 jam selepas suntikan radiofarmaseutikal. Tiada peningkatan bilangan lesi tumor positif yang signifikan secara statistik yang dikesan pada skan tertunda 3 jam. Hasil kajian ini juga

menunjukkan bahawa terdapat peningkatan kadar SUVmax bagi lesi tumor positif antara skan pada 1 jam dan 3 jam Ga-68 PSMA PET CT ($p < 0.001$ menggunakan ujian Mann-Whitney-U) dengan kadar tertinggi didapati di bahagian metastasis tulang. Nilai PSA bagi skan positif didapati lebih tinggi berbanding skan negatif Ga-68 PSMA (min PSA 5.11 ng/mL vs 2.82 ng/mL) tetapi tidak signifikan ($p = 0.17$ menggunakan ujian Mann-Whitney-U test). Tahap PSA median adalah 3.1 ng/mL dengan hanya 46% pesakit mempunyai tahap PSA < 3.0 ng/mL. Kadar SUVmax dan PSA telah menunjukkan korelasi yang signifikan dalam pengesanan lesi tumor.

KESIMPULAN : Ga-68 PSMA PET/CT menunjukkan kadar pengesanan lesi tumor prostat positif yang tinggi. Secara umumnya, kajian ini mendapati nilai pengimejan tertunda Ga-68 PSMA PET/CT bagi mengesan kanser prostat berulang adalah terhad. Walaubagaimanapun, terdapat peningkatan kadar SUVmax bagi majoriti lesi tumor positif dengan pengimejan tertunda. yang didapati membantu dalam kes-kes tertentu bagi mengesahkan lesi tumor positif melalui peningkatan kadar SUVmax.

ABSTRACT

PURPOSE: Prostate specific antigen (PSA) levels and imaging modalities serve as a tool to detect recurrent prostate cancer after radical prostatectomy or radiation treatment for localized prostate cancer. Gallium-68 (Ga-68) prostate specific membrane antigen (PSMA) positron-emission tomography/computed tomography (PET/CT) has shown high detection rate in primary staging and recurrent prostate cancer with significant clinical impact on treatment allocation. However, Ga-68 PSMA PET/CT has shown limitation in delineating benign and malignant prostatic tissue due to physiological variability, tracer accumulation and post treatment changes within the pelvic region. The aim of this study is to evaluate the value of delayed imaging in Ga-68 PSMA PET/CT to detect prostate cancer lesion in biochemically recurrent patients.

METHODS: This retrospective analysis included 87 biochemical recurrent prostate cancer patients with detectable or rising PSA level after radical prostatectomy or radiation therapy. Imaging consisted of whole-body PET/CT at 1 hour post injection (1 h p.i) and 3 hour post injection (3 h p.i). The early 1 h p.i and 3 h p.i Ga-68 PSMA PET/CT images were analyzed visually and semi quantitatively. The number of detected lesions maximum standardized uptake value (SUVmax) were recorded for analysis.

RESULTS: Ga-68 PSMA PET/CT showed overall high detection rate of 84%. A total of 158 PSMA positive lesions were identified in 73 out of 87 patients. The standard 1 h p.i. scan can detect majority of lesions with sufficient tracer uptake and optimum target to background contrast for qualitative assessment of malignant lesions. No additional lesion was detected at 3 h p.i. The mean PSA of the positive scans was higher than that of negative scans (mean PSA 5.11 ng/mL vs 2.82 ng/mL) however was not statistically significant ($p = 0.17$ by Mann-Whitney U-test). Our data demonstrated a statistically significant higher SUVmax in most pathologic lesions between 1 h p.i. and 3 h p.i. scans

($p < 0.001$ by Wilcoxon Signed Ranks Test in local recurrence, pelvic and extra pelvic nodes and bones) except for soft tissue organ metastasis ($p = 0.110$ by Wilcoxon Signed Ranks Test). Only 46% of patients with PSA level < 3.0ng/m had PSMA positive scans. There was a weak correlation observed between the PSA level and the SUVmax of the PSMA avid lesions between early and delayed imaging.

CONCLUSION: Ga-68 PSMA PET/CT showed high detection rates in biochemical recurrent prostate cancer. The value of routine whole body delayed Ga-68 PSMA PET/CT in detection of lesion in biochemical recurrent prostate cancer is limited in terms of detecting higher number of PSMA lesions. However, the overall increased in the SUVmax in the delayed imaging than 1 hour p.i imaging. for majority of tumor localization is still helpful to clarify equivocal lesions seen at standard 1 hour imaging based on the SUVmax.

INTRODUCTION

1. INTRODUCTION

Prostate cancer is the second most commonly diagnosed cancer in men worldwide (Bray et al., 2018). Patients affected by prostate cancer are treated with radical prostatectomy or external beam radiotherapy (EBRT). Despite high successful treatment rates, relapses can be seen in up to two-third of patients within 10 years of initial therapy (Ceci et al., 2015). A detectable or raising PSA after initial therapy suggests tumour recurrence. Apart from serum PSA, imaging studies also play an important role in the context of biochemical recurrent prostate cancer. The main goal of clinical restaging in suspected prostate cancer recurrence is to determine the presence and extent of disease for further planning of appropriate treatment. With conventional imaging modalities, CT and MRI perform equally poor in detection of nodal metastasis in this group of patients, particularly in patients with low PSA levels (Sterzing et al., 2016).

Gallium-68 labelled PSMA ligand (Ga-68 PSMA) is introduced as a target radiotracer for PET imaging of prostate-specific membrane antigen (PSMA) for prostate cancer. Ga-68 PSMA Positron Emission Tomography/Computed Tomography (PET/CT) has reported high detection rates for prostate cancer lesions in primary disease and suspected tumour recurrence. Studies with monoclonal antibody have also described increased expression of PSMA in advanced and metastatic castrate resistance prostate cancer (mCRPC). This

characteristic makes PSMA ligand valuable as an early indicator of tumour recurrence (Schmuck, Nordlohne, et al., 2017).

The recommended acquisition protocol for detection of prostate cancer lesion using Ga-68 PSMA PET/CT is imaging at 60 minutes after tracer injection (Fendler et al., 2017). However, there are limitations and pitfalls of Ga-68 PSMA PET/CT in the assessment of prostate cancer which include prostatic and non-prostatic benign or malignant uptake, and variability in physiological and post-therapy uptakes. These limitations have led to several Ga-68 PSMA PET/CT imaging protocols being practiced including early dynamic imaging of the prostate, delayed-time-point imaging and administration of drugs to enhance clearance of tracer. At present, our centre utilises the additional delayed-time-point imaging protocol to improve the detection of Ga-68 PSMA lesions. Therefore, the purpose of this study is to assess the value of delayed imaging of Ga-68 labelled PSMA PET/CT in the detection of recurrent prostate cancer.

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. EPIDEMIOLOGY OF PROSTATE CANCER

Prostate cancer is the second most commonly diagnosed cancer and one of the leading causes of death in men worldwide (Bray et al., 2018). For a disease as common as prostate cancer, relatively little is known about its aetiology. The highest prostate cancer incidence rates are found in the African descent in the United States and Caribbean suggesting that ethnicity and genetic predisposition are risk factors for prostate cancer. A family history of prostate cancer is a well-established risk factor with greater than two-fold risk of death from the disease. Hereditary prostate cancer is associated with earlier age of diagnosis and onset of disease. However, the aggressiveness of the disease and the clinical course do not seem to vary with the rest of the population except the carriers of the rare BRCA2 germline abnormality (Ferlay et al., 2013). Other than genetic factor, a wide variety of exogenous risk factors have been linked with an increased risk of prostate cancer such as dietary intake, inflammation, sexual behaviour, and low ultraviolet exposure (Arnold et al., 2015).

In Malaysia, prostate cancer is the 5th most common cancer in men in the age of 60 to 74 years old (10.4 %) and 75 years old and above (14.5 %) after lung and colorectal cancers (Manan et al., 2016). In our multi-ethnic population, the highest incidence rates are found in the Chinese followed by Indian and Malay. Up to 60 % of Malaysian men are diagnosed with advanced stage disease (stages 3 and 4) at initial presentation (Manan et al., 2016).

In certain areas of the world, there is a significant increase in the incidence of prostate cancer after the introduction of prostate specific antigen (PSA) screening in the late 1980s. This has led to earlier prostate cancer diagnosis, improved treatment, and a decrease in mortality rate seen in countries such as the Northern America, Oceania, Northern and Western Europe, and developed countries of Asia.

In contrast, there is a rising mortality rate seen in several South American, Asian, and Central and Eastern European countries, including Malaysia. In these countries, the mortality rate is increasing up to 6.6 per 100 000 of the population (Saad et al., 2019). Some of the factors that may partly contribute to this trend include increase in male life expectancy, a more westernized lifestyle, and limited access to effective treatment (Bray et al., 2018).

2.2. DIAGNOSTIC EVALUATION OF PROSTATE CANCER

2.2.1. Screening and early detection

Before the introduction of prostate-specific antigen (PSA), prostate cancer is suspected by physically examining a patient with a digital rectal examination (DRE). At early stage of the disease, a patient is often asymptomatic or starts to have lower urinary tract symptoms once the tumour compresses the adjacent structures (urethra, sphincter, or neurovascular bundle). These symptoms are similar to benign prostatic hypertrophy. By the time the patient is presented for DRE, often the detected cancer has already spread (Lucia et al., 2004). Abnormal DRE finding suggestive of prostate cancer includes asymmetry, nodularity, hard consistency, and loss of median groove. DRE has a low positive predictive values between 5 and 30 % in patients with low PSA level with high false positive rates (Bois et al., 2020).

PSA is a better predictor of prostate cancer than DRE. Without PSA screening, many cases of prostate cancer do not become clinically evident. PSA screening for prostate cancer targets men at higher risk of prostate cancer which include men aged between 55 and 69 years old, or with family history of prostate cancer, and BRCA 1/2 carriers. The European screening trial (ERSPC) shows a 25 % relative reduction in prostate cancer mortality in the screening arm after a median follow-up of 16 years (Schröder et al., 2014).

The higher PSA level indicates greater likelihood of prostate cancer. Elevated PSA levels more than 20 ng/mL are associated with high-risk prostate cancer. However, biopsy-proven high grade cancers can still be detected even in men with normal PSA levels of 4.0 ng/ml or less (Lucia et al., 2004).

An abnormal PSA and/or DRE is followed by histopathological verification of prostate tissue using transrectal ultrasound (TRUS)-guided biopsy (at least 10 to 12 core biopsies) taken bilaterally. The 2014 International Society of Urological Pathological (ISUP) modified Gleason scoring system is the recommended grading system for prostate cancer. The prostate biopsy foci are reported based on the location and five histological growth patterns based on the Gleason scoring system. The score is yielded from the sum of the most extensive pattern (primary) plus the second most (secondary) pattern seen on the prostate tissues' biopsies (Mottet et al., 2017). Gleason 1 represents the best differentiated pattern with the most favourable outcome, whereas Gleason 5 is the least differentiated pattern associated with aggressive disease and poor outcome (Mottet et al., 2017). As shown in Table 2.2, the 2014 ISUP grading system limits the number of prostate cancer grades, ranging them from 1 to 5.

Table 2.2. 2014 International Society of Urological Pathology (ISUP) Gleason score and Gleason grade groups.

Gleason Grade Group	Gleason score	Definition
1	< 6 (3+3 or 3+2 or 2+3 or 2+2)	Only individuals with discrete well-formed glands.
2	7 (3+4)	Predominantly well-formed glands with lesser component of poorly/fused/cribiform glands.
3	7 (4+3)	Predominantly poorly formed/fused/ cribiform glands with lesser component of well-formed glands.
4	8 (4+4 or 3+5 or 5+3)	Only poorly formed/fused/ cribiform glands (>85 %) or predominantly well-formed glands and lesser component lacking glands or predominantly lacking glands and lesser component of well-formed glands.
5	9-10	Lack of gland formation (or with necrosis) (>95 %) with or without formed/fused/ cribiform glands.

Note: Adapted from N. Mottet (2017) EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local treatment with curative intent (European Urology (2017); Volume 71, issue 4 pp 618-629).

2.3. CLASSIFICATION AND STAGING SYSTEM

The Gleason score system is a powerful tool to predict prognosis in prostate cancer patients. It has been incorporated into AJCC (American Joint Committee on Cancer) for Tumour, Node, Metastasis (TNM) classification for staging and EAU risk group classification system as shown in Table 2.3 and Table 2.3.1.

Table 2.3: Clinical Tumour, Node, Metastasis (TNM) Classification of Prostate Cancer

T - Primary Tumour (stage based on digital rectal examination [DRE] only)		
TX	Primary tumour cannot be assessed	
T0	No evidence of primary tumour	
T1	Clinically inapparent tumour that is not palpable	
	T1a	Tumour incidental histological finding in 5% or less of tissue resected
	T1b	Tumour incidental histological finding in more than 5% of tissue resected
	T1c	Tumour identified by needle biopsy (e.g. because of elevated prostate-specific antigen [PSA])
T2	Tumour that is palpable and confined within the prostate	
	T2a	Tumour involves one half of one lobe or less
	T2b	Tumour involves more than half of one lobe, but not both lobes
	T2c	Tumour involves both lobes
T3	Tumour extends through the prostatic capsule	
	T3a	Extracapsular extension (unilateral or bilateral)
	T3b	Tumour invades seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, levator muscles, and/or pelvic wall	
N - Regional (pelvic) Lymph Nodes[1]		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Regional lymph node metastasis	
M - Distant Metastasis[2]		
M0	No distant metastasis	
M1	Distant metastasis	
	M1a Non-regional lymph node(s)	
	M1b Bone(s)	
	M1c Other site(s)	

[1] Metastasis no larger than 0.2 cm can be designated pNmi.

[2] When more than one site of metastasis is present, the most advanced category is used. (p)M1c is the most advanced category.

Note: Adapted from N. Mottet (2017) EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local treatment with curative intent (European Urology (2017); Volume 71, issue 4 pp 618-629).

The EAU risk group classification system incorporates the TNM stage T-score, PSA level, and Gleason score, and stratifies prostate cancer patients into those with low, intermediate, or high risks. This risk classification system has been adapted in the National Comprehensive Cancer Network (NCCN) guidelines to guide further staging work-up and treatment decisions (Mottet et al., 2017). It also provides valuable prognostic information by predicting disease recurrence.

Table 2.3.1 EAU risk groups for biochemical recurrence of localized and locally advanced prostate cancer

Definition			
Low-risk	**Intermediate-risk**	**High-risk**	
PSA <10 ng/mL	PSA 10-20 ng/mL	PSA >20 ng/mL	any PSA
and GS <7 (ISUP grade 1)	or GS 7 (ISUP grade 2/3)	or GS > 7 (ISUP grade 4/5)	Any GS (any ISUP grade)
and cT1-2a	or cT2b	or cT2c	cT3-4 or cN+
Localized			Locally advanced

Note: Adapted from N. Mottet (2017) EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis and Local treatment with curative intent (European Urology (2017); Volume 71, issue 4 pp 618-629).

2.4. PROSTATE CANCER TREATMENT

Radical prostatectomy and external beam radiotherapy (EBRT) are considered the primary local treatments for localized, non-metastatic prostate cancer. The goal of treatment with radical prostatectomy is to eradicate prostate cancer as much as possible, while preserving pelvic organ functions such as incontinence and potency (Mottet et al., 2017). In individuals with risk of nodal metastasis, radical prostatectomy with pelvic lymph node dissection (PLND) is indicated.

Another definitive treatment for localised prostate cancer is EBRT. In the article by Mottet *et al.* (2017), randomised controlled trials (RCTs) have shown significant improvement in 5-year biochemical-free survival in men with intermediate or high-risk prostate cancer who underwent EBRT in escalated dose range of 74 to 80Gy. A combination of short- or long-term androgen deprivation therapy (ADT) is offered to selected patients with locally advanced prostate cancer to prevent risk of recurrence outside the irradiated volume (Mottet et al., 2017).

In advanced prostate cancer , the mainstay treatment includes radiotherapy to the primary tumour combined with systemic therapy such as docetaxel, arbiterone or enzalutamide (Parker et al., 2020).

2.5. BIOCHEMICAL RECURRENCE (BCR) IN PROSTATE CANCER

Despite high rates of successful initial treatment for localised prostate cancer, a substantial number of prostate cancer patients develop biochemical recurrence within 10 years after radical prostatectomy or radiation therapy (Ceci et al., 2015; Eissa et al., 2018).

Biochemical recurrence is a term used to described patients with localised prostate cancer with increasing serum PSA level after initial therapy. Evaluation of biochemical recurrence includes PSA monitoring at least every 6 months up to 5 years. These patients do not have objective evidence of distant metastatic disease at initial diagnosis.

Biochemical recurrence following radical prostatectomy is defined as increasing serum PSA trend or serum PSA value of equal or more than 0.2ng/ml. In patients who receive EBRT, biochemical recurrence is defined as increasing serum PSA trend or an increase in PSA value of 2 ng/ml above nadir (Eissa et al., 2018).

Based on a previous epidemiological study, Eissa *et al.* (2018) highlight that biochemical recurrence is followed by clinical recurrence within 8 years after initial therapy. Up to one-third of these patients progress to metastatic disease and the most common site of recurrence involves the skeletal bone (42.1 %). Based on the "seed and soil" theory proposed by Paget's, the prostate cells need to settle for an appropriate microenvironment. Under the influence of various signalling molecules, prostate cells preferentially migrate to bone, promoting osteolytic and osteoblastic lesion, and eventually proliferate in the bones, particularly in the axial skeleton (Wong et al., 2019). The second most common sites of recurrences are observed in the prostatic bed and lymphatic tissue (30.5 %), followed by retroperitoneal region (13.7 %) and lungs (Eissa et al., 2018).

In biochemical recurrence, histopathology confirmation is not always feasible and can subject to sampling bias due to small volume disease. Therefore, once PSA relapse is noted, imaging plays an important role to identify the anatomical site of recurrence (local

or disseminated disease), and plan for further appropriate treatment. For instance, salvage radiotherapy can be considered in suspected local recurrences in the prostatic fossa. However, for suspected systemic relapse, treatment with 5α-reductase inhibitors, chemotherapy, and radionuclide therapy should be considered in carefully selected patients (Mottet et al., 2011).

2.6. THE ROLE OF IMAGING IN PROSTATE CANCER

The 2015 NCCN Guidelines for Prostate Cancer recommends the current standard imaging modalities such as computed tomography (CT), multiparametric MRI (mp-MRI), and radionuclide bone scintigraphy in the evaluation of prostate cancer. The guidelines state that pelvic CT or MRI should be used in all patients with T3-T4 disease or those with T1-T2 disease with nodal involvement. Radionuclide bone scintigraphy is recommended to stage patient with PSA levels > 20ng/ml, Gleason score >>8, T3-T4 disease, or history of symptoms to suggest possible bone metastasis.

Patients with biochemical recurrence after radical prostatectomy or EBRT are usually asymptomatic until clinical manifestation precedes by 7 to 8 years. In recurrent prostate cancer, these conventional imaging modalities have limited ability to detect local and distant metastases early due to its poor specificity especially at low serum PSA level (Afshar-Oromieh et al., 2017b).

CT and MRI have been used to detect locoregional nodal metastasis in the pelvic and retroperitoneum. However, both modalities are mainly based on morphological criteria and have poor performance to detect recurrent disease particularly in small-volume diseases (Hövels et al., 2008).

In either CT or MRI, the lymph node involvement depends solely on the size and shape, rather than the actual tumour involvement. A threshold of 10mm on short axis nodal measurement is labelled as abnormal node in morphological imaging. Yet, there is a significant number of metastatic nodes which are sub-centimetre in measurements (Hövels et al., 2008).

In an article review, Beresford *et al.* (2010) describe CT to be positive up to 14 % in men with mean PSA value of 12.4 ng/mL. It is also highlighted that even in histologically-proven pelvic recurrence, only one-third of patients show positive findings on pelvic CT that is suggestive of recurrence in post-radical prostatectomy group (Beresford et al., 2010).

On MRI, each positive lesion seen is evaluated using PI-RADS scoring system which has a high negative predictive value ranging from 63 % to 98 % (Mottet et al., 2017). The utility of MRI has improved with the use of endorectal coil and additional acquisition techniques. Dynamic contract-enhanced MRI (DCE-MRI) and diffusion weighted imaging MRI (DWI-MRI) techniques enable the evaluation of vascular flow and permeability, and these techniques have been correlated with a better tumour localisation and lymph node involvement for local staging in prostate cancer. However, in biochemical recurrence, Beresford *et al.* (2010) highlight that DCE-MRI has a sensitivity and specificity of 71 % and 94 %, respectively, to detect recurrence in high-risk group of prostate cancer after radical prostatectomy.

The NCCN guidelines recommend radionuclide bone scintigraphy for staging and restaging patients with PSA levels > 20ng/ml, Gleason score >>8, T3-T4 disease, or history of symptoms to suggest possible bone metastasis. In a study of 113 patients, Lengana *et al.* (2018) reported the detection rate of bone metastasis using bone scintigraphy was less than 5 % for PSA values below 7 ng/mL. The same study has also highlighted that 54 % of the missed lesions on Tc-99m bone scintigraphy were marrow or lytic bone metastases (Lengana et al., 2018). Fluorine-18 sodium fluoride (F-18 NaF) PET/CT is getting increasing interest due to its superior spatial resolution, and it is more sensitive and specific in detecting bone metastasis.

In the last decade, molecular imaging technique using PET radiopharmaceuticals has been studied in prostate cancer. Along with F-18 NaF, radiolabelled choline (either as F-18 flurocholine, F-18 FCH or C-11 choline) PET/CT has been proven to be a better diagnostic tool for restaging biochemical recurrent prostate cancer compared to conventional imaging. A review article highlights that the radiolabelled choline derivatives demonstrate a high positive detection rate that correlates with the PSA and Gleason score, and has a significant clinical impact on treatment planning (Giovacchini et al., 2017). However, C-11 choline also lacks sensitivity at low PSA levels (Ceci et al., 2015).

Due to these limitations, the prostate specific membrane antigen (PSMA) has recently gained prominence as a molecular target for clinical imaging of prostate cancer.

2.7. PROSTATE-SPECIFIC MEMBRANE ANTIGEN

Prostate-specific membrane antigen (PSMA) was identified in the 1990s. PSMA is an integral membrane protein of the prostate epithelium with both intracellular and extracellular domains. It consists of an 18-aminoacid intracellular domain, 25-amino acid transmembrane region, and a 707 amino acid extracellular portion, and also possesses several enzymatic properties including glutamate-preferring carboxyl peptidase (Shetty et al., 2018) .

The effects of these enzymatic functions are not fully understood, however it has been shown that PSMA triggers intracellular signals to allow internalization of the protein in the external surface into the endosomal compartment (Shetty et al., 2018).

PSMA is expressed on the epithelial surface of the prostatic ducts in both benign and malignant prostate epithelial cells. Dysplastic changes of the prostate result in the expression of protein on the surface of the prostatic cells. Current immunohistochemical studies have described variable extent and intensity of cytoplasmic immunoreactivity of PSMA in both the benign and neoplastic diseases. Although benign high-grade prostatic hyperplasia exhibits increased PSMA expression, PSMA is upregulated by manyfold in prostate adenocarcinoma and its metastases (Ceci et al., 2015). In a study by Bostwick *et al.* (1998), intense cytoplasmic immunoreactivity of PSMA can be observed in every prostatic tissue, however, the intensity and number of immunoreactive cells increased from benign epithelium to high-grade prostatic intraepithelial neoplasia (PIN) to prostate adenocarcinoma. Within the adenocarcinoma group, they reported that the most intense and extensive staining was seen in cases of Gleason 4 and Gleason 5, dominant pattern which showed staining in most of the cells. Whereas in Gleason 3, there was greater

heterogeneity of staining observed, particularly in areas of low- or intermediate-grade adenocarcinoma (Bostwick et al., 1998).

Previous studies with monoclonal antibody have shown that expression of PSMA level increased with tumour dedifferentiation, in metastatic, advanced, and castrate resistance prostate cancer (Giovacchini et al., 2017). Regardless of androgen status, PSMA was also upregulated following androgen deprivation state (Shetty et al., 2018).

Given the significantly higher PSMA expression in nearly all tumour stages of prostate cancer, those characteristics make PSMA valuable as an early indicator of disease recurrence and prognostication (Schmuck, Nordlohne, et al., 2017).

2.7.1. PSA NEGATIVE PROSTATE CANCER

As previously mentioned, a biopsy-proven high-grade prostate can still be detected even in men with normal PSA levels of 4.0 ng/ml or less (Lucia et al., 2004). Yang and colleagues of Harvard Medical School have analysed the data of half a million of cases of men diagnosed with clinical T1 to T4N0 in the epidemiology SEER program. They highlighted that 1 % of prostate cancer cases had negative or very low PSA level than the actual tumour burden (Yang et al., 2017).

Without a detectable or high surrogate PSA levels, detecting and establishing diagnosis using imaging modalities were crucial in this group of PSA-negative high-risk prostate cancer patients. This group of patients might present clinically symptomatic of metastasis with similar disease pattern with the high-grade, high-PSA patients.

The low-PSA, high-grade prostate cancer also had higher mortality rate (2.15 times higher) compared to high-risk, high-PSA valued prostate cancer. Despite the low serum PSA levels, the disease can be an aggressive disease with poor prognostication due to delay in diagnosis and treatment (Yang et al., 2017).

Birtle *et al.* (2005) highlighted a marked prostate specific membrane antigen (PSMA) and androgen receptor immunostaining which can be seen in up to 90 % of PSA-negative, high-risk group of patients. Given the high expression of PSMA immunostaining, serum and tissue of PSMA levels can be utilised to evaluate this group of patients. One of the possible explanation that may explain the heterogeneity in PSMA and PSA level in prostate cancer include neuroendocrine trans-differentiation of the prostate cells (Damjanovic et al., 2018).

2.8. GALLIUM-68 PROSTATE-SPECIFIC MEMBRANE ANTIGEN

A German group developed a small receptor ligand for PSMA known as Ga-68-HBED-CC PSMA or Ga-68 PSMA-11 (Afshar-Oromieh et al., 2016). This new class of radiotracer specifically targeted the large extracellular domain of the PSMA transmembrane protein that was highly expressed on the prostatic cells (Giovacchini et al., 2017).

The radionuclide Gallium-68 was obtained from a Ge-68/Ga-68 radionuclide generator and was used for radiolabelling of PSMA-11. Ga-68 PSMA pharmacokinetics with half-life of 68 minutes, beta decay of 189keV, and availability of Ge-68/Ga-68 generator made it suitable for application in everyday clinical practices. The number of studies describing the value of Ga-68 PSMA ligand PET/CT has been reported increasingly in the evaluation

of primary disease staging and restaging of biochemical recurrences in prostate cancer. In a large meta-analysis involving 1309 patients, Perera *et al.* (2016) stated the sensitivity and specificity of Ga-68 PSMA-11 PET/CT were 80 % and 97 %, respectively, based on per lesion analysis. Another study by Rauscher *et.al.* (2016) compared the Ga-68 PSMA PET/CT findings with histological findings of lymph nodes in biochemical recurrent prostate cancer post salvage lymphadenectomy. The Ga-68 PSMA PET/CT showed a sensitivity of 77.9 % compared to only 26.9 % of CT and MRI in detecting nodal metastasis

Ga-68 PSMA PET/CT has also been reported to change the treatment plan of biochemically recurrent prostate cancer. The impact of Ga-68 PSMA PET/CT on treatment was assessed in a prospective study of biochemical recurrent patients who were scheduled to receive salvage RT or ADT. This study reported up to 40 % of cases had the treatment allocation changed after Ga-68 PSMA/PET CT, for example, extending salvage RT to metastases and replacing salvage RT with systemic therapy (Bluemel et al., 2016).

2.9. IMAGING OF GA-68 PSMA PET/CT

Based on the available evidence, the use of Ga-68 PSMA PET/CT in biochemical recurrent prostate cancer varies between institutions.

The 2017 Joint European Association of Nuclear Medicine (EANM) and The Society for Nuclear Medicine and Molecular Imaging (SNMMI) highlight a few clinical applications of Ga-68 PSMA PET/CT which include:

i. localization of tumour tissue in recurrent prostate cancer;
ii. primary staging of high-risk disease before surgical procedures or planning EBRT;
iii. staging before or during PSMA-directed radiotherapy; and
iv. targeted biopsy after previous negative biopsy and monitoring of systemic treatment response in metastatic disease (Fendler et al., 2017).

Ga-68 PSMA PET/CT acquisition protocol involves a CT scan performed from the vertex to mid-thigh followed by PET acquisition (Fendler et al., 2017). On PET images, the accumulation and distribution of Ga-68 PSMA ligand in cells are linked to the epithelial expression of PSMA in various tissues. The prostate cancer lesion usually appears as a focal tracer uptake with strong tumour to background ratio compared to the surrounding tissue (Fendler et al., 2017). Since the antigen is not specific to prostate tissues, Ga-68 PSMA uptake can be observed in other tissues with PSMA expression such as the lacrimal, salivary, spleen, and kidneys as shown in Figure 2.9.

An intense tracer uptake can also be observed in the renal pelvis, ureters, and urinary bladder due to route of excretion (Shetty et al., 2018). This can result in difficulty to delineate between the pathological lesion and the physiological uptake of adjacent structures in the pelvis (e.g. seminal vesicles, lymph nodes) (Hövels et al., 2008).

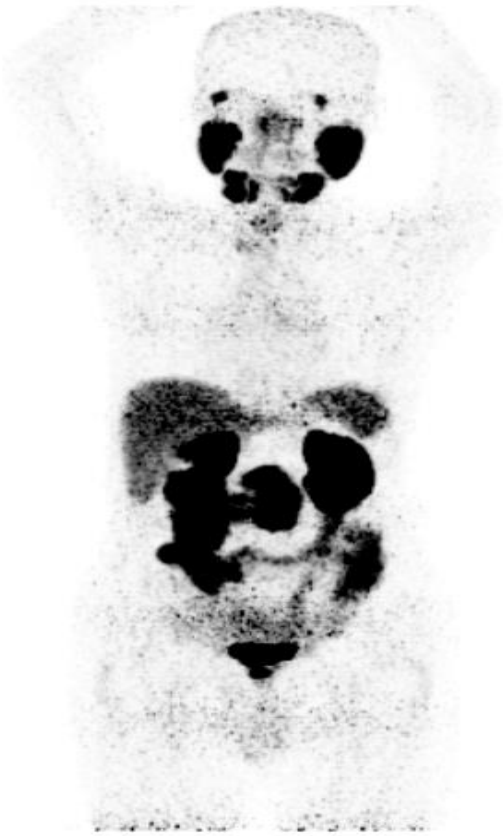

Figure 2.9: Normal physiological distribution in Ga-68 PSMA PET scan on maximum intensity projection (MIP) images.

For Ga-68 PSMA PET/CT, the SNMMI guidelines recommend an interval uptake time between 50 and 100 minutes, and delayed imaging up to 3 to 4 hours after injection of radiotracer. A previous study has shown that Ga-68 PSMA uptake in malignant lesions increased over time with better target to background contrast (Kumar et al., 2018). The three-hour (h) post injection (p.i.) scan helps to identify lesions within close proximity to the route of tracer excretion in the ureter or the bladder, or lesions with a low PSMA expression and slower accumulation of tracer (Rauscher et al., 2016). A study done by Afshar-Oromieh *et al.* (2017) reported that delayed imaging at 3 h p.i might help to further clarify unclear lesions, and reduced the rate of false negative findings of other benign and malignant pathologies (Afshar-Oromieh et al., 2017b).

There are also a few recent publications that have evaluated multiple time-point imaging with dynamic acquisitions as early as 3 to 10 minutes post tracer injection to enhance the specificity of the Ga-68 PSMA PET imaging. Schmuck *et al.* (2017) reported up to 95 % of primary prostate lesions can be identified on early dynamic images and continue to show increasing tracer uptake until 3 h p.i.. The addition of an early image would also increase the detection of malignant lesions in the pelvis that may be masked by the urinary system activity (Ceci et al., 2015).

The activity of Ga-68 PSMA in PET imaging can also be analysed with a semi-quantitative method using Standardised Uptake Value (SUV) (Fendler et al., 2017). SUV is measured by uptake normalisation as a fraction of the injected dose per weight, over the entire body density (in mg per mL units) (Thie, 2004). A previous study by Uprimny *et al.* (2017) has observed higher SUVmax values in patients with lymph node metastases compared to patients without malignant nodal involvement. The intensity of the Ga-68 PSMA also correlated with the Gleason score and PSA levels.

JUSTIFICATION

AND BENEFIT