Anna Maria Ringheim

**QUANTIFICATION OF THE PHARMACOKINETICS OF ^{68}Ga -PSMA-11 IN
PROSTATE CANCER PATIENTS USING HYBRID POSITRON
EMISSION TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING**

Thesis presented at Faculdade Israelita de
Ciências da Saúde Albert Einstein for the
degree of Doctor of Philosophy in Health
Sciences.

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Dedication

To Jeferson, Cecilia, Camilla and Carolina for your love and
patience.

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List of abbreviations and symbols

3D	Three dimensional
^{11}C	Carbon-11 isotope
^{18}F	Fluorine-18
^{68}Ga	Gallium-68 isotope
^{68}Ge	Germanium-68 isotope
μm	Micrometer
ADC	Apparent diffusion coefficient
Ahx	Aminohexanoic acid
AIF	Arterial input function
AUC	Area under the curve
BBB	Blood-brain barrier
C_1	Activity concentration in the first tissue compartment
C_2	Activity concentration in the second tissue compartment
C_P	Activity concentration in arterial plasma
C_T	Activity concentration in tissue
CT	Computed tomography
C_{VOI}	Activity concentration in PET image VOI
C_{WB}	Activity concentration in arterial whole blood
DCE	Dynamic contrast-enhanced
DWI	Diffusion-weighted imaging
EDTA	Ethylenediaminetetraacetic acid
FDG	Fludeoxyglucose
h	Hour
HASTE	Half-Fourier single shot turbo spin echo
HBED-CC	N,N'-bis [2-hydroxy-5-(carboxyethyl)benzyl] ethylenediamine-N,N'- diacetic acid
HPLC	High-performance liquid chromatography
ICC	Intraclass correlation coefficient
IDIF	Image-derived input function
K_1	Rate constant from blood compartment to tissue compartment
k_2	Rate constant from tissue compartment to blood compartment

k_3	Rate constant from first to second tissue compartment
k_4	Rate constant from second to first tissue compartment
kDa	Kilodalton
kg	Kilogram
K_i	Influx rate $K_1k_3/(k_2+k_3)$
MBq	Mega Becquerel
mg	Milligram
min	Minute
MIP	Maximum intensity projection
mL	Milliliter
MR	Magnetic resonance
MWCO	Molecular weight cut-off
OSEM	Ordered-Subsets Expectation Maximization
PET	Positron emission tomography
PI-RADS	Prostate Imaging - Reporting and Data System
PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
PVE	Partial volume effect
Q1	Quartile 1
Q3	Quartile 3
RC	Recovery coefficient
RF	Radio-frequency
s	Second
SD	Standard deviation
SNR	Signal-to-noise ratio
SPECT	Single Photon Emission Tomography
SUV	Standardized uptake value
SUV_{max}	Maximum standardized uptake value
T2WI	T2 weighted image
TAC	Time-activity curve
TFA	Trifluoroacetic acid
TNM	Tumor node metastasis
TOF	Time-of-flight
UHPLC	Ultra-high performance liquid chromatography

UV	Ultraviolet
V_B	Fractional blood volume
VIBE	Volumetric interpolated breath-hold examination
VOI	Volume of interest
V_T	Total distribution volume

Abstract

Introduction: Positron emission tomography with the radiopharmaceutical Glu-NH-CO-NH-Lys(Ahx)-HBED-CC labeled with ^{68}Ga (^{68}Ga -PSMA-11), targeting the prostate-specific membrane antigen, has demonstrated great potential for detection and staging of prostate cancers. **Purpose:** To quantify ^{68}Ga -PSMA-11 uptake by pharmacokinetic modeling using arterial blood sampling in primary prostate cancer patients, and to assess the use of simplified methods for quantification. **Methods:** Fifteen patients with histologically proven primary prostate cancer underwent a 60-minute dynamic ^{68}Ga -PSMA-11 positron emission tomography scan of the pelvis with axial T1 Dixon, T2 and diffusion-weighted magnetic resonance images acquired simultaneously. Arterial blood activity was measured by automatic arterial blood sampling and manual blood samples were collected for plasma-to-blood ratio correction and for metabolite analysis. Time-activity curves were derived from volumes of interest in lesions, normal prostate and muscle, and mean standardized uptake values calculated. ^{68}Ga -PSMA-11 kinetics were assessed by pharmacokinetic compartment modeling and the model selection based on the Akaike information criterion. Image-derived input functions using a magnetic resonance-guided method were obtained and evaluated as an alternative to arterial blood sampling. Kinetic parameters were correlated to standardized uptake values, patient data and magnetic resonance parameters. **Results:** In total 18 positive lesions on positron emission tomography and magnetic resonance were identified. ^{68}Ga -PSMA-11 was stable *in vivo*, not requiring metabolite correction. The kinetics could be described by an irreversible two-tissue compartment model. The kinetic rate constants K_1 and k_3 and the net influx rate constants K_i were all significantly higher in lesions compared with normal tissue ($p < 0.05$). K_i derived using blood sampler and image-derived input showed excellent agreement with an intraclass correlation coefficient of 0.99. The K_i showed the strongest correlation with SUV of time-window 30-45min ($\rho = 0.95$, $p < 0.001$). Both K_i and standardized uptake values correlated significantly with serum prostate specific antigen level and prostate specific antigen density. **Conclusions:** The ^{68}Ga -PSMA-11 kinetics can be described by an irreversible two-tissue compartment model. An magnetic resonance-guided image-derived input function can be used as a non-invasive alternative to blood sampling. Standardized uptake

values showed strong correlation with K_i and can be used in clinical practice to quantify ^{68}Ga -PSMA-11 uptake.

Keywords: Prostatic neoplasms. Positron-emission tomography. Magnetic resonance imaging. Pharmacokinetics.

1 INTRODUCTION

Cancer of the prostate, a gland that is part of the male reproductive system and responsible for producing the semen fluid, is the second most frequently diagnosed malignant neoplasm in men worldwide, with more than one million new cases estimated per year.⁽¹⁾ Higher risk of developing prostate cancer has been linked to age, ethnic group, genetic and lifestyle factors.⁽²⁾ There is also a geographic variation in the diagnosis of new cases, which in part reflects the more widespread use of prostate-specific antigen (PSA) testing in developed countries as compared to developing countries.⁽²⁾

The most common form of prostate cancer is adenocarcinoma, which is the cancer affecting the gland cells of the prostate. The prostate is divided into three zones: peripheral zone, where most of the gland cells are located, transition zone and central zone. More than 70% of all prostate cancers arise in the peripheral zone of the prostate.⁽³⁾

1.1 Diagnosis of prostate cancer

Prognostic staging usually follows the American Joint Committee on Cancer Prognostic Staging system, which combines the information from TNM classification, Gleason histological grading and serum PSA level.^(4,5) The TNM classification is based on digital rectal examination, imaging techniques or radical prostatectomy specimen, where T refers to the size and extent of the primary tumor, N to the extent of spread to nearby lymph nodes in the pelvis and M to the presence of metastasis in distant parts of the body, usually distant lymph nodes and bones.⁽⁴⁾ The Gleason score is the histological grade of abnormal tissue in the tumor, sampled using transrectal ultrasound-guided biopsy. It consists of a grade from 1 to 5 of the predominate pattern and of the secondary pattern in the tumor tissue sample, resulting in a combined grade of 2 to 10, for example 3+4 or 4+4.⁽⁶⁾ The International Society of Urological Pathology proposed a new grading system based on the Gleason score: Grade Group 1 = Gleason score ≤ 6 , Grade Group 2 = Gleason score $3 + 4 = 7$, Grade Group 3 = Gleason score $4 + 3 = 7$, Grade Group 4 = Gleason score 8 and Grade Group 5 = Gleason scores 9 and 10.⁽⁷⁾ The serum PSA test is a widely used screening

test, which measures the blood concentration of PSA, a protein produced by the prostate gland cells and found to be elevated in prostate cancers.⁽⁸⁾ A correct diagnosis of the disease stage is essential to provide the patient with the appropriate treatment option, which include active surveillance, surgery, radiation, hormone, chemo- or immunotherapy.⁽⁹⁾

Imaging has an important role in disease staging and detection of tumor recurrence after radical prostatectomy. Multiparametric MR imaging combines at least two MR sequences to improve accuracy, where the most common combination is T2 weighted sequence (T2WI), which is the primary sequence for the transition zone of the prostate, and diffusion-weighted imaging (DWI), which is the primary sequence for the peripheral zone. Adding dynamic contrast-enhanced (DCE) imaging might improve the detection rate further, and can provide information on tissue perfusion and permeability.^(10,11) DWI is based on the molecular diffusion of water molecules in the tissues. From DWI an apparent diffusion coefficient (ADC) can be derived, which has been shown to correlate negatively with Gleason score.⁽¹²⁾ Analysis of multiparametric MR imaging of the prostate can be used as a screening tool in patients with clinical suspicion for prostate cancer from elevated serum PSA and/or altered digital rectal examination of the prostate. The MR image analysis usually follows the PI-RADS v2 (Prostate Imaging - Reporting and Data System, version 2) classification, where a score is given on a scale from 1 to 5, where 1 being most probably benign and 5 being highly suspicious of malignancy.⁽¹³⁾

1.2 Positron emission tomography

Positron emission tomography (PET) is a molecular imaging technique for quantitative measurement of physiological parameters *in vivo*. The first PET scanners were introduced in the 1960's and have ever since undergone huge improvements, as increased spatial resolution and sensitivity and reduced scan time.⁽¹⁴⁾ In 2019, the first human study using a total body PET scanner demonstrated that a whole-body PET acquisition as short as 1min or less could produce PET images of diagnostic quality.⁽¹⁵⁾ PET imaging is based on the detection of picomolar quantities of biological substances, specific to the molecular target of interest (metabolic process, receptor, molecular pathways, etc.), which have been labeled with a positron-emitting

radioisotope. As the unstable radioisotope decays, it emits a positron, which travels a few millimeters through the tissue before it encounters an electron. This annihilation event results in a pair of 511 keV energy photons, emitted in opposite directions. The detector ring of the PET camera detects these photons in coincidence, therefore the high sensitivity in comparison to other conventional nuclear medicine techniques as Single Photon Emission Tomography (SPECT).⁽¹⁶⁾ The principal limitations of PET are the relatively low spatial resolution and the low signal-to-noise ratio (SNR).⁽¹⁷⁾ The low spatial resolution results in partial-volume effects (PVE), which affect quantification of small lesions. Some PET scanners utilize the time-of-flight (TOF) technology, which improves the localization accuracy of the annihilation event. Time-of-flight does not directly lead to improved spatial resolution, but it does increase the SNR and thereby the image quality and lesion detection.⁽¹⁷⁾ Since around year 2000, practically all PET scanners are hybrid, either combined with CT or more recently with MR for co-registered anatomical data and for attenuation correction.⁽¹⁸⁾

Positron emission tomography/Computed tomography with the glucose analog Fludeoxyglucose labeled with the radioisotope ^{18}F (^{18}F FDG) has an established role in clinical practice for diagnosis, staging, restaging and treatment monitoring of several tumor types. However, for prostate cancer, it presents limited sensitivity.⁽¹⁹⁾ The PET radiopharmaceutical Choline, labeled with ^{11}C or ^{18}F , has been studied extensively in primary and recurrent prostate cancer patients, but studies have shown only moderate sensitivity, especially at low PSA levels.⁽²⁰⁻²²⁾

A new radiopharmaceutical, labeled with ^{68}Ga and targeting the prostate specific membrane antigen (PSMA), has recently become to play an important role in the detection, diagnosis and staging of both primary and recurrent prostate cancers.

1.3 Prostate specific membrane antigen

Prostate-specific membrane antigen is a transmembrane protein with higher expression in prostate cancer cells in comparison with normal prostate cells.⁽²³⁾ A shorter spliced version of PSMA, called PSM', is expressed in normal prostate cells, where it is localized primarily in the cytoplasm, but its function is unknown.⁽²⁴⁾ During the transformation of prostate tissue cells to cancerous cells, PSMA is transferred from the internal side of the plasma membrane to the outer side. Studies have shown that as the

cancer progresses, the ratio PSMA/PSM' increases.⁽²⁴⁾ The transmembrane dimeric form of PSMA has carboxypeptidase enzymatic activity.⁽²⁵⁾ When a molecule, for example an inhibitor, binds to PSMA, the molecule is internalized and is retained in the lysosomes or released in the cytoplasm.⁽²⁶⁾ This is especially interesting for imaging purposes, as the radioligand would internalize and increase its deposition in the cell, thereby improving tumor-to-background contrast.⁽²⁷⁾

There are several antibodies targeting the extracellular domain of PSMA,⁽²⁶⁾ but their use as PET radiopharmaceuticals is limited due to their slow clearance from the bloodstream resulting in high background uptake.⁽²⁷⁾ Recently, the specific substrate recognition site of PSMA was identified as composed of two structural motifs: a lipophilic pocket and a motif interacting with urea-based inhibitors.⁽²⁸⁾ After this important discovery, a number of ligand and inhibitor molecules have been developed to target the PSMA protein, both for therapy but particularly for diagnostic imaging. The most studied molecule is the urea-based PSMA inhibitor peptide Glu-NH-CO-NH-Lys(Ahx)-HBED-CC, which, labeled with ⁶⁸Ga, serves as a PET radiopharmaceutical (here called ⁶⁸Ga-PSMA-11).^(29,30) It has a high affinity and specificity for PSMA and, due to its fast internalization when binding to human prostate adenocarcinoma cells, accumulates fast even in small lesions.⁽³⁰⁾ Studies have reported non-prostate-related PSMA expression in normal tissue, as in liver, spleen, small bowel, urinary tract and salivary glands,^(31,32) but also in other non-prostate malignant tissues as breast carcinoma, osteosarcoma, adrenal adenoma, glioblastoma, renal cell carcinoma and non-small cell lung carcinoma.⁽³³⁾ In these malignancies, PSMA expression usually occurs in endothelial cells of tumor-associated neovasculature.⁽³³⁾ In prostate cancer, the metastasis are usually located in lymphatic tissue and bones, hence the normal tissue uptake does not usually interfere with image reading.⁽³⁴⁾

⁶⁸Ga-PSMA-11 consists of the hydrophilic pharmacore Glu-NH-CO-NH-Lys, which specifically interacts with the extracellular active binding site of PSMA and of the lipophilic acyclic Ga(III) chelator N,N'-bis [2-hydroxy-5-(carboxyethyl)benzyl]

ethylenediamine-N,N'- diacetic acid (HBED-CC), which binds to the lipophilic pocket of the PSMA binding site. Ahx, or aminohexanoic acid, serves as a spacer between the pharmacore and the chelator (Figure 1). Comparisons to other chelators as the DOTA conjugate, has shown that HBED-CC has superior thermodynamic stability, higher specific internalization in prostate adenocarcinoma cells and reduced unspecific binding.⁽³⁰⁾

^{68}Ga -PSMA-11 consists of a ^{68}Ga – chelator HBED-CC and the motif responsible for the specific binding to the PSMA transmembrane protein
 Source: Adapted from Eder M, Schäfer M, Bauder-Wüst U, Hull WE, Wängler C, Mier W, et al. ^{68}Ga -complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjug Chem.* 2012;23(4):688–97.⁽³⁰⁾
Figure 1. Chemical structure of ^{68}Ga -PSMA-11

A series of studies using ^{68}Ga -PSMA-11 PET/CT and PET/MR have demonstrated its great potential for detection and staging of primary and recurrent prostate cancers.⁽³⁴⁻⁴¹⁾ The combination of multiparametric MR and ^{68}Ga -PSMA-11 PET results in increased detection rate in comparison with MR alone.⁽⁴²⁾ Also, the combination of PET and MR enables precise targeted fusion biopsy to obtain histological confirmation of the tumor.^(36,39) Several studies have demonstrated higher sensitivity and specificity of ^{68}Ga -PSMA-11 PET for lymph node and bone metastasis staging in comparison with standard imaging techniques.^(27,43-46) Detection of recurrent prostate cancer, especially at low serum PSA levels, is currently a challenge due to the limited detection rate of conventional imaging techniques. Studies have shown a superior detection rate of suspicious lesions by ^{68}Ga -PSMA-11 PET in comparison with MR, CT, choline PET and bone scintigraphy.^(35,38,42,47,48)

1.4 Quantification in positron emission tomography

In clinical practice, the most commonly used method for PET quantification is the standardized uptake value (SUV). Its calculation is simple and routinely available in commercial imaging software packages. SUV is a semi-quantitative measure of the total radioactivity concentration in a region of interest at a time point of the PET scan normalized by the injected activity and the subject's weight.

There are several advantages of using SUV in clinical practice. Firstly, it does not require a long dynamic scan, but only enough time per bed position to produce images with sufficient radioactive counts/quality to enable diagnostic reading. The images are acquired after a predefined time post-injection to allow the radiopharmaceutical to distribute in the body. Secondly, the calculation of SUV does not depend on measurement of radiopharmaceutical concentration in blood, which makes the study less invasive for the patient and less expensive. Thirdly, its calculation is simple and routinely available in commercial imaging software packages. However, studies have shown that SUV may differ between sites and scanners, when not controlling for image acquisition parameters such as image acquisition post tracer injection, reconstruction algorithms and attenuation and scatter corrections.⁽⁴⁹⁾ We recently performed a study comparing the reproducibility of maximum standardized uptake values (SUV_{max}) between ^{68}Ga -PSMA-11 PET/CT and PET/MR scans.⁽⁵⁰⁾ To reduce the influence of ^{68}Ga -PSMA-11 uptake as a function of time, half of the patients acquired PET/CT first, followed by PET/MR and the other half PET/MR first, followed by PET/CT. We showed that SUV_{max} from PET/CT and PET/MR were linearly correlated, but on average 20% higher on PET/CT than on PET/MR and should therefore not be compared without applying a correction factor.

The most accurate method to quantify PET data is pharmacokinetic compartment modeling.⁽⁴⁹⁾ Compartment modeling assumes that tracer kinetics can be separated into tissue compartments, which are connected to an arterial blood compartment (Figure 2). The tracer exchanges between blood and tissue compartments by certain rate constants, which can be determined by a set of linear, first-order differential equations. The arterial blood compartment is represented by the arterial input function: the concentration of the radiopharmaceutical in arterial plasma. The total tissue radioactive concentration is derived from a region of interest of the PET image. The most common compartment models are the irreversible and reversible one-tissue and two-tissue-compartment models (Figure 2). The derived rate constants can be used to estimate biologically relevant measures of interest, as blood flow, binding potential of a ligand to its receptor and metabolic rate of glucose. There are simplifications to the compartment model analysis, where the most common are the graphical Patlak and Logan methods.^(51,52) The Patlak model assumes that when all reversible compartments are in equilibrium with plasma, the Patlak plot becomes linear and the positive slope represents the net influx rate K_i . In the Logan plot, the linear slope represents the total

distribution volume (V_T) of the radiopharmaceutical in tissue. The advantages of the graphical methods are less sensitivity to data noise and faster and easier calculation in comparison to the iterative compartment model fits. The main disadvantage is that they do not provide the individual compartment rate constants, but only the macroconstants K_i or V_T . The kinetic models for some radiopharmaceuticals used in oncology have been established, for example ^{18}F FDG⁽⁵²⁾ and ^{18}F -Choline⁽⁵³⁾. Both are well described by an irreversible model.

Kinetic modeling analysis enables quantification of PET pharmaceutical uptake, independent of scan time post-injection and blood plasma clearance. The kinetic modeling, however, requires dynamic PET scanning and arterial blood data, which makes it less time-efficient and more invasive in comparison to SUV analysis. Simplified analysis methods, as SUV, are adopted in clinical practice, but their correlation to compartment modeling, should first be validated.⁽⁴⁹⁾

Irreversible one-tissue compartment model (A), reversible one-tissue compartment model (B), irreversible two-tissue compartment model (C) and reversible two-tissue compartment (D). C_p denotes the concentration in arterial plasma, C_T the total tissue concentration, C_1 the concentration in the first tissue compartment, usually free radiopharmaceutical in tissue, and C_2 the concentration in the second tissue compartment, usually specifically bound. The rate constants K_1 , k_2 , k_3 and k_4 indicate the rate of exchange between compartments.

Source: Adapted from Tomasi G, Turkheimer F, Aboagye E. Importance of quantification for the analysis of PET data in oncology: review of current methods and trends for the future. *Mol Imaging Biol.* 2012;14(2):131–46.⁽⁴⁹⁾

Figure 2. Positron emission tomography pharmacokinetic compartment models

The arterial input function (AIF), which is the concentration of radiopharmaceutical in arterial plasma as a function of time, is the input to the compartment model analysis and its precise determination is essential in PET quantification. Ideally, the activity concentration in arterial blood should be determined using an online arterial blood sampling system, which measures the concentration in high temporal resolution through a coincidence counter. This allows the exact determination of the peak of the radiopharmaceutical bolus injection. Since the online detector measures only the activity in whole blood, it is necessary to take manual samples during the PET scan and process these samples to determine the activity in plasma.

In the body, PET radiopharmaceuticals, as any other molecule or drug, may undergo biotransformation reactions leading to other chemically different species (metabolites), whereas the amount of parent compound decreases. However, as PET only detects radioactive decay, in PET modeling our only concern is with radiolabeled metabolites.⁽⁵⁴⁾ The AIF should reflect the concentration of only parent compound in arterial plasma. Therefore, plasma samples should be analyzed by high-performance liquid chromatography (HPLC) to separate the radiolabeled metabolites from the parent compound. From the chromatogram, the amount of parent compound is estimated as a fraction of total plasma activity.

Due to the invasive blood sampling, alternative kinetic modeling approaches have been proposed, as the use of venous blood, radiopharmaceutical infusion to reach steady state, PET image reference tissue region or the use PET image-derived blood to substitute blood sampling. However, Oertel et al.⁽⁵⁵⁾ compared the use of alternative approaches to invasive arterial blood sampling and concluded that none of the proposed methods could provide an accurate blood concentration curve necessary for the full compartment modeling analysis. Methods to extract blood information from the PET image provide an attractive alternative to arterial blood sampling, as they noninvasively obtain the arterial blood curve during the PET scan. For whole-body PET, most methods outline volumes of interest (VOIs) over the common iliac artery of the PET image to extract the blood data. These methods, however, usually suffer from partial volume effects due to limited spatial resolution of the PET image. With a combined PET/MR scanner, the high-resolution MR image can be used to outline the arteries and when combined with partial volume correction approaches,⁽⁵⁶⁾ provide a more accurate blood input function for kinetic modeling.⁽⁵⁷⁾

Previously published studies on the biodistribution and pharmacokinetics of ^{68}Ga -PSMA-11 in prostate cancer patients reported increasing SUV in tumor lesions from early to late imaging and rapid blood clearance of ^{68}Ga -PSMA-11.⁽⁵⁸⁻⁶⁰⁾ Pfoh et al.⁽⁵⁸⁾ acquired whole-body PET/MR images at 10 min, 60 min, 130 min, and 175min after administration of ^{68}Ga -PSMA-11 in 5 patients. The assessment of the biodistribution showed high accumulation in kidneys, liver, salivary glands and spleen. The study showed that ^{68}Ga -PSMA-11 has rapid blood clearance and high rate of renal excretion. As to the dosimetry, the urinary bladder wall received the largest absorbed dose, followed by the kidneys.

There is not yet any consensus on the optimal time-window to acquire ^{68}Ga -PSMA-11 PET images, but the most recent guideline recommends a 60min uptake time of ^{68}Ga -PSMA-11 and whole-body and pelvic images starting at 50-100min after ^{68}Ga -PSMA-11 administration are common in clinical routine.^(34,60-62) Although these late images ensure sufficient time for tracer concentration in the neoplastic tissue, progressive urinary bladder accumulation may impair imaging interpretation.

Sachpekidis et al. published two studies on the pharmacokinetics of ^{68}Ga -PSMA-11, one in primary prostate cancer and one in recurrent prostate cancer, acquiring 60-min dynamic PET data.^(59,60) The authors used PET image-derived blood as input to the compartment model analysis, and omitted correction for plasma-to-blood ratio and study of labeled metabolites in plasma. The data were analyzed with a reversible two-tissue compartment model to estimate the rate constants K_1 , k_2 , k_3 and k_4 , as well as the fractional blood volume V_B and the net influx rate K_i . SUV correlated strongly with K_i , moderately with serum PSA and weakly with Gleason score from biopsy.^(59,60) To date, full kinetic modeling with arterial blood sampling and metabolite analysis has not yet been reported, nor has the use of simplified methods as SUV and image-derived blood data been assessed.

Hospital Israelita Albert Einstein was one of the first sites outside of Europe to produce and clinically investigate ^{68}Ga -PSMA-11 and the first site in Latin America to install and operate a hybrid PET/MR scanner.

1.5 Purpose

1. Quantify the kinetics of ^{68}Ga -PSMA-11 using pharmacokinetic compartment modeling;
2. Assess the use of semi-quantitative methods for quantification of ^{68}Ga -PSMA-11 uptake;
3. Assess the use of image-derived blood as an alternative to arterial blood sampling;
4. Assess correlation between positron emission tomography quantitative and semi-quantitative parameters and multiparametric magnetic resonance, serum prostate-specific antigen and histopathological data.

2 METHODS

2.1 Patients

Fifteen patients were included in this observational prospective study. The study was approved by the Ethics Committee of Hospital Israelita Albert Einstein and informed consent was obtained from all individual participants. Inclusion criteria were histologically proven primary prostate cancer (Gleason score ≥ 6), with a tumor size of at least 0.6cm in diameter on MR imaging and ability to remain supine for at least one hour. Exclusion criteria were multiple malignancies, anticoagulant therapy and contraindications to MR imaging, such as claustrophobia and MR-incompatible implants. Serum PSA had been measured previously and the prostate weight had been estimated by a previous MR scan. PSA density was calculated as serum PSA divided by the prostate weight. The equipment used in the study are listed in appendix 1.

2.2 Positron emission tomography/magnetic resonance imaging

The radiopharmaceutical ^{68}Ga -PSMA-11 was synthesized with a specific activity of approximately 40 MBq/ μg at the radiopharmacy laboratory of Hospital Israelita Albert Einstein according to previous publications.⁽³⁰⁾ The patients underwent a 60-minute dynamic PET/MR scan in list-mode with the field of view over the pelvic area. After a short break to empty the bladder, the patients underwent a whole-body PET/MR scan from skull base to mid-thigh for clinical purposes. Both scans were acquired on a Biograph mMR scanner (Siemens, Germany) with arms-down. At the start of the first PET scan, a bolus injection of ^{68}Ga -PSMA-11 (median 191.6 MBq, range 103.9 - 231.3 MBq) was manually administered intravenously and flushed with 40 mL of saline.

We used a 24-channel spine radiofrequency (RF) coil integrated within the MR bed and three surface body coils (6 channels each) to cover the thorax, abdomen and pelvis. The following MR data were acquired during both PET scans: axial T1 volumetric interpolated breath-hold examination (VIBE) Dixon (18sec), axial T2 half-Fourier single shot turbo spin echo (HASTE) without fat suppression with breath-holding (23sec) and axial diffusion-weighted imaging without breath-holding (2min 19sec).

Positron emission tomography data were reconstructed with a 3D-Ordered-Subsets Expectation Maximization (OSEM) algorithm (3 iteration, 21 subsets,

matrix 256x256, 4mm Gaussian filter) and corrected for decay, scatter and attenuation using Dixon-based MR sequences. The list mode data were reconstructed into 28 time frames (10 x 30s, 5 x 60s, 5 x 120s, and 8 x 300s), with shorter time frames in the beginning of the scan to capture the blood to tissue kinetics of ^{68}Ga -PSMA-11 after bolus injection.

2.3 Image analysis

The reconstructed PET/MR images were analyzed by one board-certified nuclear medicine physician and one radiologist using the software syngo.via (Siemens, Germany) regarding number and types of lesions. Quantitative analysis included outlining of VOIs on the images to derive time-activity curves (TACs) in unit Bq/mL.

Tumor lesion TACs were derived from VOIs applied using isocontour threshold of 40% of maximum SUV on late PET images (45-60min post-injection) with the location confirmed on the T2-weighted MR images. Spherical VOIs of 1mL were outlined on normal prostate and gluteus muscle to derive normal tissue TACs.

Magnetic resonance images were analyzed regarding lesion size, PI-RADS v2 score⁽¹³⁾ and ADC.

Standardized uptake value in unit g/mL, normalized to body weight, were calculated using the following formula:

$$SUV = \frac{C_{PET}(t)}{dose / weight}$$

Where $C_{PET}(t)$ is the decay corrected activity concentration in kBq/mL at time point t, dose is the injected activity in MBq and weight is the patient weight in kg. Mean SUV, which is the average SUV within a volume, was calculated for all VOIs.

For a subpopulation of 12 patients, we evaluated lesion detectability as a function of time. One subject was excluded due to shorter PET scan and two subjects were excluded due to technical problems with the PET image reconstruction. The dynamic PET list mode data were grouped into four static images (0-15, 15-30, 30-45 and 45-60 minutes post-injection). These images were analyzed by two experienced nuclear medicine physicians regarding number and types of lesions and divergences were solved by consensus.

2.4 Arterial input function

Arterial blood activity concentration as a function of time was determined by sampling from a cannula inserted into the radial artery of the patients. The arterial puncture was done by an anesthesiologist using local anesthesia and then connected to pressurized saline. Due to the position of the arm inside the PET/MR scanner, an extension line connected the radial artery and the access for blood sampling. To capture the sharp ^{68}Ga -PSMA-11 bolus peak, during approximately the first 10min activity was measured using an automatic blood sampling device with 1s temporal resolution (twilite, swisstrace GmbH, Switzerland), connected to a shielded pump (B Braun Infusomat Space, flow rate 4.17 mL/min for the first 7min, then 2.5mL/min for the remaining 3min). Previously, the blood sampling device had been calibrated to the PET/MR scanner, by scanning a PET phantom filled with a solution containing a known concentration of ^{18}F FDG and simultaneously measuring a catheter filled with the same solution in the twilite device.

At 6 time points (approximately 3, 7, 15, 25, 40 and 60min post-injection) manual arterial samples (4.9 mL) were collected in Ethylenediaminetetraacetic acid (EDTA) treated tubes. At time points 3 and 7min, the automatic blood sampling was briefly paused to collect the manual samples. Before each manual blood sample, a sample containing mainly saline was collected and discarded to make sure the real sample contained only blood. When the pump was disconnected, the pressurized saline prevented the catheters from clotting. The blood sampler curve was corrected for decay, background and dispersion, and resampled to 5 sec time frames between 0 and 5min, and 15 sec time frames for the remaining time using the PSAMPLE software (PMOD, Switzerland).

The manual blood samples were immediately put on ice. From the whole-blood sample, a 500 μL volume was separated and the radioactivity measured (100s) in a gamma counter (Triathler, HIDEX, Finland). The remaining whole-blood sample was centrifuged at 5,000 x g for 3min at 4°C (Sorvall ST 16 centrifuge, Thermo Scientific, USA) to separate the plasma. A 500 μL volume of plasma was separated and the radioactivity measured in the gamma counter. The gamma counter had been calibrated previously to the PET/MR scanner. Plasma-to-blood activity ratios were calculated from these samples.

The blood sampler curve was merged with the decay-corrected manual whole blood samples to get a 60-min AIF using an in-house Matlab program. This whole-blood AIF was then converted into a plasma AIF using the average plasma-to-blood activity ratios from the manual samples for each subject.

2.5 Metabolite analysis

The plasma samples were analyzed for possible labeled metabolites. We compared two different protocols.

In the first protocol, we used precipitation of proteins to prepare the sample for ultra-high performance liquid chromatography (UHPLC) analysis. An equal volume of acetonitrile was added to the plasma volume to precipitate the plasma proteins and was then centrifuged at 5,000 x g for 6min at 4°C to separate the supernatant from the plasma proteins. The supernatant was transferred on ice to another laboratory, located a 15min drive away, where the samples were analyzed by an UHPLC system, equipped with a radioactivity detector. First, the samples were centrifuged again at 14,000 x g for 12min at 4°C (Centrifuge 5804 R, Eppendorf, Germany) to assure that there were no plasma proteins present in the sample and filtered using a 22µm syringe filter. The supernatant was then analyzed as described below.

The alternative protocol was applied on the plasma samples from the last two patients, due to a suspicion that the first protocol was not adequate. Here, the plasma samples were filtered using an Amicon Ultra-0.5mL centrifugal filter (Merck Millipore, Germany) with a molecular weight cut-off (MWCO) of 3kDa. This filter purified the proteins with a molecular weight below 3,000 Da (molecular weight of ⁶⁸Ga-PSMA-11 is 1,014 Da), from the larger plasma proteins. The filters were first washed once with 500µL of NaOH (50mM) and then five times with 500µL of purified water MilliQ using centrifugation at 20,000 x g for 10min. The plasma was filtered by centrifugation at 20,000 x g for 10min at 4°C. The supernatant was then filtered using a 22µm syringe filter and analyzed as described below.

The supernatant from either sample preparation protocol was analyzed using a 1290 Infinity II UHPLC system (Agilent Technologies, USA) equipped with a radioactivity detector (Eckert & Ziegler, Germany). A volume of 100µL was loaded using automatic injection. The samples were maintained at 4°C. The analytical

column was a Phenomenex Kinetex® reverse-phase C18 (100mm x 3mm; 2.6µm), maintained at 25°C with water + 0.1% trifluoroacetic acid (TFA) (mobile phase A; 95%) and acetonitrile + 0.1% TFA (mobile phase B; 5%) at flow rate 0.8mL/min. Gradient of mobile phase B was: 5-10% (0.0-0.5min); 10-30% (0.5-7.0min); 30-5% (7.0-7.5min); 5% (7.5-15.0min). The flow rate was kept at 0.8mL/min.

Due to the low concentration, ^{68}Ga -PSMA-11 could not be detected neither by the ultraviolet (UV) detector, nor by the radioactivity detector. We therefore collected UHPLC fractions every 20s, which were counted (100 s) in an automatic gamma counter (Wizard 2TM 3" 2480, Perkin Elmer, USA). Measured radioactivity was plotted as a function of time and compared to the retention time of a standard solution containing ^{68}Ga -PSMA-11, analyzed using the same chromatographic protocol. The fraction intact ^{68}Ga -PSMA-11 was determined from the UHPLC radiochromatogram.

To determine its stability *in vitro*, an aliquot of 19 MBq of ^{68}Ga -PSMA-11 was incubated in 1mL of plasma for 60min at 37°C and analyzed using the same protocol.

2.6 Image-derived input function

The image-derived input functions (IDIF) were generated in collaboration with the researcher Udunna Anazodo at Lawson Health Research Institute, Canada, using an MR-guided method.⁽⁵⁷⁾ The IDIF were generated by extracting the median PET activity within a vessel mask at each time frame. The vessel mask was defined by segmentation of both external iliac arteries clearly visible on Dixon-MR sequence registered to PET (Figures in appendix 2). The IDIF were corrected for spillover of PET activity from surrounding tissue to the arteries and partial volume effect (PVE) (by recovery coefficients (RC)) using the approach described in Croteau et al.⁽⁶³⁾ Because the PVE and spillover corrections were estimated based on measurements made previously using ^{18}F and highly dependent on the size of the vessel, the vessel mask was restricted to a segment of each iliac artery where the vessels were relatively straight and had the largest diameter (8-9mm). The IDIF was then converted to plasma curves using the average plasma-to-blood ratio determined from the arterial blood sampling. In total, IDIF were derived for 12 subjects. One subject was excluded due to shorter PET scan, which did not provide data enough for fitting of the method and two subjects were excluded due to excessive motion, which resulted in

difficulties to extract the IDIF. The ratio of the area under the curve (AUC) between IDIF and whole blood AIF ($AUC_r = AUC_{IDIF} / AUC_{AIF}$) was calculated to verify equivalency of the two input functions.

2.7 Pharmacokinetic analysis and model validation

Four different compartment models were fitted to the data: an irreversible one-tissue compartment model with rate constant K_1 (1T1k), a reversible one-tissue compartment model with rate constants K_1 and k_2 (1T2k), an irreversible two-tissue compartment model with rate constants K_1 , k_2 and k_3 (2T3k) and a reversible two-tissue compartment model with rate constants K_1 , k_2 , k_3 and k_4 (2T4k) (Figure 2). The model equations are listed in appendix 3. Physiologically, in the two-tissue compartment model, K_1 and k_2 would represent the transport of ^{68}Ga -PSMA-11 from arterial blood to tissue and back to venous blood. The rate constants k_3 and k_4 would represent the binding/unbinding of ^{68}Ga -PSMA-11 to the PSMA transmembrane protein. If the exchange of tracer between the first and second tissue compartments is fast enough that the two cannot be distinguished kinetically, the two tissue compartments may be joined into one compartment that represents both the free and specifically bound tracer within the tissue, i.e. the simpler one-tissue compartment model.^(53,64) To account for the contribution from blood activity to the tissue TACs, all models were assessed with and without the fractional blood volume V_B . When V_B was accounted for, it was fitted together with the rate constants. The model that best described the lesion uptake was selected based on the Akaike information criterion (AIC),^(65,66) which is a data-driven approach to model selection. The net influx rate $K_i = K_1 * k_3 / (k_2 + k_3)$ was calculated from both the irreversible two-tissue compartment model and from the Patlak graphical method.⁽⁵²⁾

As blood input to the pharmacokinetic models, we compared AIF plasma curves and IDIF plasma and whole blood curves. Analysis was done using an in-house Matlab program (Mathworks, version 2018b).

The net influx rate K_i was correlated to patient, MR and histopathological data.

2.8 Semi-quantitative analysis

Simplified protocols and methods for quantification are preferred in clinical settings. Therefore, SUV of static images from time-windows 15-30min, 30-45min and 45-60min were calculated and correlated to the net influx rate K_i and to patient, MR and histopathological data.

2.9 Statistical analysis

Statistical analysis was performed using software R (version 3.4.1, R Core Team (2017)),⁽⁶⁷⁾ with packages.^(68,69) The level of significance was set to 5%.

Categorical variables were described by absolute frequency and in percentage. The distributions of all numerical variables were visually checked and tested for normality and described accordingly by mean $\pm$ standard deviation (SD) or by median values with 25th and 75th percentiles (Q1 and Q3) and plotted in boxplots.

Differences between tissue regions and scan time-windows were tested with the Friedman test. Correlations between patient characteristics, MR parameters, SUV and kinetic modeling parameters, as well as between K_i from the compartment and Patlak models, were assessed using Spearman correlation analysis (ρ). The agreement between K_i using AIF and IDIF was determined using intraclass correlation analysis (ICC3, two-way, mixed model). The agreement between K_i from the compartment model and graphical Patlak analysis, as well as between K_i calculated using AIF and IDIF were visualized in Bland-Altman plots with 95% limits of agreement (LOA). For lesion-based analysis, the most avid lesion (highest SUV in the last time frame of the scan) per subject was selected to avoid accounting for dependency between multiple lesions from a single subject.

3 RESULTS

3.1 Patient and imaging characteristics

The 15 patients included in the study had a median age of 67 years (Q1=62.00, Q3=72.50) and a median body weight of 75.00kg (Q1=71.00, Q3=84.50).⁽⁷⁰⁾ The median serum PSA level was 8.64ng/mL (Q1=5.31, Q3=11.77) with a median PSA density of 0.21ng/mL/g (Q1=0.12, Q3=0.43). The median injected activity was 191.55 MBq (Q1=176.89, Q3=213.68) and the mean injected activity per body weight was 2.52 ± 0.67 MBq/kg. Table 1 summarizes the patient and imaging characteristics.

One patient had its scan interrupted at 40min, due to excessive movement. Only lesions identified on both the PET and MR images were considered. One patient (number 13) was undergoing external-beam radiotherapy of the prostate and had no significant lesion uptake in the PET image. This patient was excluded from further PET image analysis. Of the 14 ⁶⁸Ga-PSMA-11 positive patients, 10 had one lesion and four patients had two lesions. In total 18 lesions were identified with a median lesion size of 1.80cm (Q1=1.10, Q3=2.75), of which 17 were located in the prostate and one identified as a lymph node metastasis. The Gleason scores from previous biopsies were between 6 and 10, with a median Gleason score of 7.00 (Q1=7.00, Q3=7.00). The PI-RADS score for the lesions located in the prostate was between 2 and 5, with a median PI-RADS score of 5.00 (Q1=4.00, Q3=5.00). The median ADC was $0.71 \times 10^{-3} \text{ mm}^2/\text{s}$ (Q1= 0.61×10^{-3} , Q3= 0.95×10^{-3}). Figure 3 shows typical ⁶⁸Ga-PSMA-11 PET/MR images and figure 4 the TAC's for all subjects. ⁶⁸Ga-PSMA-11 showed increasing accumulation during scan time in lesions, although at different rates. In normal prostate and muscle the washout was faster. Some curves were noisy due to low injected activity and to movement during the PET scan.

As to the lesion detectability as a function of PET scan time post-injection, the earliest images (0-15min) showed a lower detectability rate (82.4%) compared with the later images starting at 15, 30 and 45 minutes. These all had a detectability rate of 94.1%, corresponding to 16 of 17 lesions correctly identified. As expected, urinary bladder accumulation increased during scan time, with moderate or intense activity in all images starting at 30 or 45 minutes. The failure of correct lesion detection in these images was due to the interference of bladder activity (Figure 5).

Table 1. Patient and imaging characteristics

Patient no.	Age (years)	Body weight (kg)	PSA (ng/mL)	PSA density (ng/mL/g)	Injected activity (MBq)	Injected activity/body weight (MBq/kg)	Type of lesion	Lesion size on MR (cm)	Gleason score	PI-RADS score	ADC x 10 ⁻³ (mm ² /s)	SUV (45-60min)
1	77	81	97.00	2.85	103.90	1.28	PC/LN	3.3/0.9	10 (5+5)	5/NA	0.63/0.53	5.74*/13.29*
2	74	66	5.71	0.17	172.27	2.61	PC	3.5	9 (5+4)	5	0.98	6.04
3	70	75	8.64	0.12	168.74	2.25	PC	2.5	7 (3+4)	5	0.95	4.67
4	61	61	38.19	0.85	190.69	3.13	PC	1.8	7 (3+4)	5	0.71	12.05
5	74	72	5.57	0.28	231.30	3.21	PC	2.2	7 (4+3)	5	0.99	13.27
6	74	90	3.03	0.09	194.50	2.16	PC	2.0	7 (4+3)	4	0.92	5.09
7	62	74	9.11	0.25	119.40	1.61	PC	1.3	6 (3+3)	4	0.64	16.02
8	60	90	12.88	0.52	184.53	2.05	PC/PC	1.6/1.3	7 (3+4)	5/4	0.59/0.74	18.02/4.90
9	62	88	5.31	0.10	208.76	2.37	PC	0.8	6 (3+3)	4	0.95	5.92
10	71	70	10.64	0.34	227.67	3.25	PC	3.0	8 (4+4)	5	0.70	10.18
11	62	75	2.49	0.21	204.83	2.73	PC/PC	0.8/1.5	6 (3+3)	4/5	0.34/0.24	3.29/2.34
12	67	80	5.31	0.12	218.61	2.73	PC	4.6	7 (3+4)	5	0.69	11.61
13	67	59	36.00	1.00	229.25	3.89	PC	2.0	9 (5+4)	5	0.51	-
14	63	88	10.66	0.16	181.52	2.06	PC/PC	0.7/0.6	7 (4+3)	4/2	1.00/1.30	7.75/4.60
15	62	77	3.00	0.06	191.55	2.49	PC	3.0	7 (3+4)	5	0.90	5.31

PSA, prostate-specific antigen; MR, Magnetic resonance; PI-RADS, Prostate Imaging Reporting and Data System v2; ADC, apparent diffusion coefficient; SUV, mean standardized uptake value; PC, lesion located in the prostate; LN, lymph node metastasis; NA, not applicable. *SUV of time frame 30-40min.

T2-weighted axial MR image (A), averaged dynamic PET 45-60min post-injection (B), PET/MR fused image (C) and whole-body maximum intensity projection (MIP) of the pelvis (D).

Figure 3. Primary prostate cancer patient (PSA 5.57ng/mL, Gleason score 4+3, PI-RADS score 5) presenting a lesion with low signal intensity on a T2-weighted axial MR image (A) and intense focal ^{68}Ga -PSMA-11 PET uptake (B,C, D) in the left lobe of the prostate (SUV 45-60min: 13.27).

Lesions (n=18) (A), normal prostate (n=14) (B) and muscle (n=14) (C).

Figure 4. ^{68}Ga -PSMA-11 time-activity curves

PET maximum intensity projection (MIP) images showing two intense focal ^{68}Ga -PSMA-11 uptake in the left peripheral zone of the prostate (SUV 45-60min: 18.02, Gleason score 3+4, PI-RADS score 5) and in the right peripheral zone of the prostate (SUV 45-60min: 4.90, Gleason score 3+4, PI-RADS score 4). In the earliest image (0-15min post-injection), we can identify the left lesion (arrow) and no activity in the bladder (A). Images 15-30min (B) and 30-45min post-injection (C) show progressive ^{68}Ga -PSMA-11 accumulation in the bladder, but still exhibit good contrast between both lesions and urinary activity. In the late image (45-60min), delimitation of left lesion is impaired by bladder activity (D), but the right lesion does not suffer from bladder activity interference.

Figure 5. Comparison of lesion detectability as a function of time post-injection in a primary prostate cancer patient (PSA 12.88ng/mL)

3.2 Arterial input function and metabolite analysis

The activity in whole blood and plasma were counted in 83 manual blood samples. Due to blood clotting, we were not able to collect the manual samples at time points 25 and 40min in 3 patients. Due to the impossibility to reach the arterial cannula while the patient was inside the scanner, the clotted catheters could not be changed during scanning and those manual samples were lost. The mean plasma-to-blood ratio was 1.62 and stable during scan time (Figure 6).

The UHPLC analysis demonstrated that ^{68}Ga -PSMA-11 was stable both *in vivo* and incubated in plasma *in vitro*, with a retention time of 5.1min (Figure 7). The first metabolite analysis protocol using protein precipitation resulted in release of free ^{68}Ga in the supernatant, as identified by UHPLC (Figure 7). The alternative protocol using protein filtration was able to separate ^{68}Ga -PSMA-11 from the larger plasma proteins (Figure 8). Since ^{68}Ga -PSMA-11 did not present any radiolabeled metabolites

during the 60min scan time, we could consider the plasma curve as the AIF. Figure 9 shows typical AIF whole blood and plasma curves.

The dashed line indicates the overall mean plasma-to-blood ratio of 1.62 (N=83).

Figure 6. Box plot of the plasma-to-blood ratio over time

Standard solution of free ^{68}Ga with retention time of 1.1min (A). Incubated ^{68}Ga -PSMA-11 in plasma *in vitro*, processed by protein precipitation, showing a release of free ^{68}Ga (retention time 1.1min) and a smaller amount of ^{68}Ga -PSMA-11 (retention time 5.1min).

Figure 7. Radiochromatograms from metabolite analysis by UHPLC following protein precipitation method

Standard solution of ^{68}Ga -PSMA-11 with a retention time of 5.1min (A). ^{68}Ga -PSMA-11 incubated in plasma *in vitro*, showing a single peak at 5.1min (B). Patient plasma sample (25min post-injection) showing a single peak, slightly delayed due to tubing to collect UHPLC fractions (fractions collected every 20 seconds and subsequently counted in gamma counter) (C).

Figure 8. Radiochromatograms from metabolite analysis by UHPLC following protein filtration method

Figure 9. Typical blood sampler whole blood and plasma time-activity curves

3.3 Image-derived input function

The mean RC factors used for partial volume correction was 0.46 ± 0.04 and the mean spill-in factor 0.46 ± 0.03 . The resulting IDIF agreed well with the whole blood AIF with a mean AUCr of 0.84 ± 0.09 . Figure 10 shows a comparison of AIF and IDIF whole blood curves from one patient.

Figure 10. Comparison of blood sampler (AIF) whole blood and image-derived (IDIF) whole blood time-activity curves for one patient

3.4 Pharmacokinetic analysis and model validation

Based on the AIC analysis, the irreversible two-tissue compartment model without V_B correction was the model that best fitted the lesion kinetics (Figure 11). The 2T3k model was preferred in 9/18 lesions, followed by the 2T3k+ V_B (3/18), 1T2k (3/18), 2T4k (2/18) and 2T4k+ V_B (1/18). The Patlak graphical analysis showed a positive slope from 15min post-injection (Figure 11). Due to movement artifacts at the end of the scan, we excluded the last data points in the model and Patlak fits for five patients. In four lesions, the individual rate constants could not be determined with precision, but K_1 and K_i as well as Patlak K_i gave robust values. Rate constants k_2 and k_3 from these lesions were excluded from the median values (Table 2). Both K_1 , k_3 , K_i and Patlak K_i were significantly higher in lesions compared to normal prostate and muscle (Table 2 and Figure 12).

Typical ^{68}Ga -PSMA-11 time activity curves and the corresponding irreversible two-tissue compartment model fits (A). Patlak graphical analysis presented positive slopes from 15min post-injection for all regions (B). Squares: lesion; triangles: normal prostate; diamonds: muscle; solid lines: model fit.

Figure 11. Pharmacokinetic analysis of ^{68}Ga -PSMA-11

Table 2. Kinetic parameters K_1 , k_2 and k_3 , estimated by the irreversible two-tissue-compartment model (2T3k). K_i is the net influx rate calculated from rate constants $K_1 k_3 / (k_2 + k_3)$ or from the slope of the graphical Patlak analysis

Kinetic parameters	Lesion	Prostate	Muscle	p-value ^a	p-value ^b
K_1 (mL/min/mL)	0.136 [0.099, 0.229]	0.088 [0.078, 0.107]	0.028 [0.021, 0.033]	0.006	0.001
k_2 (/mL)	0.277 [0.213, 0.484]	0.239 [0.147, 0.329]	0.285 [0.184, 0.466]	0.308	0.839
k_3 (/mL)	0.108 [0.057, 0.125]	0.040 [0.036, 0.056]	0.040 [0.031, 0.045]	0.005	0.007
K_i (/mL)	0.036 [0.024, 0.076]	0.014 [0.010, 0.020]	0.003 [0.003, 0.003]	0.001	0.001
Patlak K_i (/mL)	0.035 [0.024, 0.096]	0.012 [0.008, 0.022]	0.003 [0.003, 0.003]	0.001	0.001

Median values with interquartile range [Q1, Q3], n=14 subjects, 18 lesions (median of k_2 and k_3 from 14 lesions). P-values assessed by Wilcoxon test (the most avid lesion per subject selected) comparing lesion with prostate (a) and muscle (b).

Kinetic parameters K_1 (A), k_3 (B) and K_i (C) in lesion, prostate and muscle tissues. The solid line indicates the median value.

Figure 12. Boxplots of kinetic parameters from irreversible two-tissue compartment model

K_i from the 2T3k compartment model and Patlak K_i showed strong correlation, with rho of 0.996 ($p<0.001$) in lesion, rho of 0.890 ($p<0.001$) in normal prostate and rho of 0.925 ($p<0.001$) in muscle. Bland-Altman analysis estimated a mean absolute difference of -0.006 (mean percentage difference -3.44%) between 2T3k K_i and Patlak K_i . The LOA's ranged from -0.031 to 0.018. The graph showed that for larger K_i , Patlak $K_i > 2T3k K_i$ (Figure 13).

The net influx rate constant K_i calculated using IDIF plasma showed excellent agreement with K_i derived using AIF in lesions (K_i ICC=0.99, $p<0.001$ and Patlak K_i ICC=0.98, $p<0.001$). The Bland-Altman plot showed that the mean difference between K_i (AIF) and K_i (DIF) was 0 (Figure 14). The individual rate constants K_1 and k_3 showed only moderate agreement with K_1 and k_3 using AIF in lesions (K_1 ICC=0.57, $p=0.026$, k_3 ICC=0.65, $p=0.021$). IDIF whole blood resulted in slightly weaker agreement with the AIF plasma derived rate constants (K_i ICC=0.89, $p<0.001$, Patlak K_i ICC=0.93, $p<0.001$).

As to the correlation between kinetic rate constants and patient and MR data, we found significant correlation only between K_i and PSA (rho=0.62, $p=0.018$) (Figure 15) and between K_i and PSA density (rho=0.64, $p=0.015$). The kinetic rate constants did not correlate significantly with Gleason score, PI-RADS, ADC or lesion size.

The plot indicates the mean difference between 2T3k K_i and Patlak K_i and upper and lower 95% limits of agreement.

Figure 13. Bland-Altman plot comparing K_i from the irreversible two-tissue compartment model fit (2T3k) and K_i from the graphical Patlak analysis

The plot indicates the mean difference between K_i (AIF) and K_i (IDIF) and upper and lower 95% limits of agreement.

Figure 14. Bland-Altman plot comparing K_i from the irreversible two-tissue compartment model fit using arterial input function and image-derived input function as input

Figure 15. Scatter plot of K_i from the irreversible two-tissue compartment model and serum prostate-specific antigen level.

3.5 Semi-quantitative analysis

Standardized uptake value increased significantly with time in lesion and muscle, but not in normal prostate and was significantly higher in lesion compared to normal tissue (Table 3 and Figure 16).

Table 4 summarizes the SUV correlation analysis. Lesion SUV of all time-windows correlated significantly with K_i , where SUV of time-window 30-45min showed the strongest correlation. K_i calculated using IDIF showed similar correlation coefficients as K_i calculated using AIF. SUV correlated significantly with PSA and PSA density, but not with Gleason score, PI-RADS, ADC or lesion size.

Table 3. ^{68}Ga -PSMA-11 standardized uptake value compared between regions and time-windows of dynamic scan

SUV	Lesion	Prostate	Muscle	p-value ^a
15-30min	4.95 [3.50, 6.94]	2.48 [2.05, 2.81]	0.54 [0.53, 0.56]	<0.001
30-45min	5.63 [4.04, 9.80]	2.61 [2.15, 3.12]	0.56 [0.52, 0.62]	
45-60min	5.98 [4.84, 11.72]	2.94 [2.28, 3.49]	0.61 [0.56, 0.72]	
p-value ^b	<0.001	0.063	<0.001	

Median values with interquartile range [Q1, Q3], n=14 subjects, 18 lesions. P-values assessed by Friedman test (the most avid lesion per subject selected) comparing regions (a) and time-windows (b). SUV, mean standardized uptake value.

The solid line indicates the median value.

Figure 16. Boxplot of standardized uptake value of time-windows 15-30min, 30-45min and 45-60min, respectively, for lesion, normal prostate and muscle (n=14 patients, n=18 lesions).

Table 4. Correlation between lesion standardized uptake value, net influx rate K_i calculated with arterial input function and image-derived input function, prostate-specific antigen and prostate-specific antigen density over different time-windows of the dynamic positron emission tomography scan

SUV	K_i (AIF)		K_i (IDIF)		PSA		PSA density	
	rho	p-value	rho	p-value	rho	p-value	rho	p-value
15-30min	0.83	<0.001	0.84	0.003	0.52	0.056	0.55	0.041
30-45min	0.95	<0.001	0.93	<0.001	0.64	0.014	0.61	0.019
45-60min	0.88	<0.001	0.90	<0.001	0.65	0.015	0.67	0.012

Spearman correlation rho with corresponding p-value. n=14, the most avid lesion per subject selected. Abbreviations: AIF, arterial input function; IDIF, image-derived input function; PSA, prostate-specific antigen; SUV, mean standardized uptake value.

4 DISCUSSION

The main objectives of this work were to determine the pharmacokinetics of the radiopharmaceutical ^{68}Ga -PSMA-11 in primary prostate cancer patients and to assess the use of simplified methods as IDIF and SUV for routine clinical analysis.

Based on the AIC model selection analysis and as indicated by the linear positive Patlak slope, the kinetics of ^{68}Ga -PSMA-11 during the 60-min scan time could be considered irreversible, as indicated in previous studies,^(59,60) with the irreversible two-tissue compartment model providing the best fit to the data. The irreversible kinetics of ^{68}Ga -PSMA-11 is in line with the process of internalization of PSMA ligands when binding to the specific binding site of the PSMA transmembrane protein. Internalization of PSMA ligands has been shown to be fast and practically irreversible, as the PSMA ligand is recycled in the lysosomes of the cell.⁽²⁶⁾ In the compartment model, the internalization can be represented by the rate constant k_3 , which as expected was found to be significantly higher in lesion as compared to normal prostate and muscle. K_1 , which in the kinetic model represents the rate constant of tracer transport from blood to tissue, was significantly higher in lesions than in normal prostate and muscle, which may reflect the process of angiogenesis in tumor tissue and enhanced tumor tissue permeability.⁽⁷¹⁾ If also acquiring DCE-MR, which we did not do in our study, tissue permeability as measured by MR could be correlated to K_1 , as previously done in a ^{18}F -Fluorocholine PET and DCE-MR study in prostate cancer patients.⁽⁷²⁾

The net influx rate K_i - a macroconstant, which incorporates both the transport from blood to tissue and the irreversible trapping of the tracer - was as expected also significantly higher in lesions as compared to normal prostate and muscle. Sachpekidis et al.⁽⁶⁰⁾ reported overall greater absolute values in comparison to our rate constant estimates. This could reflect a more advanced prostate cancer study population: the Sachpekidis' patient cohort had a higher median serum PSA level of 24.1 ng/mL as compared to ours of 8.6 ng/mL, but similar median age and Gleason score. Further, Sachpekidis et al. underwent their study using PET/CT, while we used PET/MR. A previous study by our group showed that PET/CT SUV_{max} were on average 20% higher than PET/MR SUV_{max} ⁽⁵⁰⁾. However, using properly calibrated PET and

arterial blood sampling devices the kinetic parameters should be less prone to PET scanner differences than SUV. More likely, the higher kinetic rate constant estimates may be the result of omitting plasma-to-blood ratio correction and the result of partial volume effects due to PET-derived blood input. This would underestimate blood activity and consequently overestimate the kinetic rate constants.

A few lesions were located close to the urinary bladder, which might have resulted in spillover of activity from bladder to lesions in the late scan times. However, as we outlined the VOIs using a threshold of 40% of maximum SUV, the surrounding activity should not have interfered largely. The location of the VOIs were also visually confirmed on the MR image. However, some of these smaller lesions might have benefitted if corrected for PVE and spillover. For some patients, the 60-min PET/MR scan was too long, which resulted in movement artifacts. This caused difficulties in fitting the compartment model to the data, but as expected the graphical Patlak method, which is less affected by noisy data, was able to estimate robust K_i values. In fact, K_i as estimated by the compartment model analysis and K_i by graphical Patlak analysis showed excellent correlation with a rho of 0.996 in lesions.

A well-determined arterial input function for kinetic modeling analysis requires the analysis of possible radiolabeled metabolites, a result of cleavage/metabolism *in vivo* of the parent molecule. PET metabolite analysis is usually developed first in an *in vitro* model, followed by a preclinical *in vivo* model and then finally applied to human studies.⁽⁵⁴⁾ We developed the metabolite analysis directly using patient samples, which is not ideal as the analysis method was developed and improved during the study. Surprisingly, the sample preparation method using acetonitrile for protein precipitation released free ^{68}Ga in the plasma samples. Acetonitrile is commonly used for sample preparation for HPLC analysis and was used by Eder et al.⁽²⁹⁾ to precipitate the proteins in plasma samples incubated with ^{68}Ga -PSMA-11 *in vitro*. Therefore, we initially interpreted the ^{68}Ga peak as a metabolite produced *in vivo*. However, after testing the alternative protocol with protein filtration, we could conclude that ^{68}Ga -PSMA-11 did not produce labeled metabolites during the 60min scan time. The reason why free ^{68}Ga was released in the first sample preparation method needs further investigation.

We showed that ^{68}Ga -PSMA-11 is stable *in vivo*, not necessitating correction for labeled metabolites. Due to the lack of metabolites, ^{68}Ga -PSMA-11 makes an ideal candidate for IDIF, only necessitating the correction for plasma-to-blood ratio.

With a plasma-to-blood ratio on average 1.62, this indicates that ^{68}Ga -PSMA-11 do not penetrate red blood cells and remains in plasma, giving a plasma-to-blood ratio (RP/B) depending only on hematocrit (HCT) following the equation $\text{RP/B} = 1/(1-\text{HCT})$. A RP/B of 1.62 would equal a hematocrit factor of 38%, which is in the lower limit of the normal range of hematocrit for men.⁽⁷³⁾ As the plasma-to-blood ratio depends only on hematocrit, the value of 1.62 should be stable between subjects, not necessitating arterial blood samples in future IDIF studies.

The lack of labeled metabolites also facilitated the pharmacokinetic model validation. As PET kinetic models were originally developed for the brain,⁽⁴⁹⁾ it is assumed there is a blood-brain barrier (BBB) between blood and tissue, which should impede hydrophilic radiolabeled metabolites to enter the tissue.⁽⁷⁴⁾ Outside of the brain, however, there is no BBB and radiolabeled metabolites could potentially enter the tissue and contaminate the true radioactivity of the parent compound in tissue. For some radioligands this can be solved by introducing an extra tissue compartment in the kinetic model to account for the activity concentration of metabolite in tissue. For ^{68}Ga -PSMA-11 the standard irreversible two-tissue compartment model provided excellent fit to the data.

The IDIF produced using the MR-guided method agreed well with the AIF, with a mean AUC ratio of 0.84. There are however some limitations to the IDIF method that should be considered. As PET was acquired during free breathing and MR during breath-hold, there is a possibility that motion could affect the accuracy of the IDIF. The vessel mask generated from MR during breath-hold may have been in a different orientation and space from the dynamic PET under free breathing, especially in the late PET frames. This we accounted for by 1) registering the MR-segmented vessel mask to the first 20 s post-injection PET frame, when the vessel activity concentration is high, and 2) using the median PET activity in the vessel VOI, instead of the mean PET activity, which is more prone to error due to motion. The London, ON, Canada site, where the IDIF were calculated, did not have access to ^{68}Ga , therefore the PVE and spillover correction experiments were performed on the same scanner model as the patient study (Biograph mMR), but using ^{18}F and ^{11}C radioactive solutions. The ^{68}Ga isotope is a less pure positron emitter than ^{18}F and ^{11}C , which can contribute to scatter and image blurring. Furthermore, in comparison to ^{18}F , ^{68}Ga is a long-range positron emitter, which contributes to loss of spatial resolution, important in partial volume error estimation.⁽⁷⁵⁾ The distance traveled by the positron can even be increased in the

presence of a magnetic field,⁽⁷⁵⁾ therefore caution should be applied to ^{68}Ga IDIF studies with small vessel sizes. In our study, the external iliac arteries were relatively large, 8-9 mm in diameter, and the effect of using ^{18}F and ^{11}C instead of ^{68}Ga for the corrections should have less impact. Despite these limitations, K_i using IDIF showed excellent agreement with the K_i using AIF with an ICC of 0.99.

Although IDIF simplifies the acquisition protocol for quantification considerably as compared to blood sampling, for routine clinical analysis there is a need for even simpler quantification methods. Here we showed that SUV of all time-windows correlated significantly with K_i , with correlation coefficients of 0.88 for K_i (AIF) and 0.90 for K_i (IDIF) in the late PET scan time-window. These are higher than the correlation coefficients of 0.76 for primary and 0.85 for recurrent prostate cancer lesions, reported in previous studies.^(59,60) Again, this might reflect the less accurate PET-derived input function as compared to our AIF and MR-derived IDIF. We report significant correlation between PSA and SUV ($\rho=0.62$), similar to the ρ of 0.60 reported by Sachpekidis et al.,⁽⁶⁰⁾ but also a significant correlation between PSA and K_i , differently from Sachpekidis et al. who did not find a significant correlation between the kinetic parameters and PSA. Our data did not, however, present a significant correlation between SUV and Gleason score, differently from Sachpekidis' study, which reported a weak but significant correlation ($\rho=0.33$, $p<0.05$). If we divide our patient cohort into high-risk patients (Gleason score $\geq 4+3$) and low-risk patients (Gleason score $\leq 3+4$), there is still no significant correlation between SUV and Gleason score. The lack of correlation between PET and MR parameters might be due to our small patient sample. Unfortunately, we were not able to obtain the radical prostatectomy Gleason scores, as not all patients had undergone surgery yet.

The strong correlation between K_i and SUV demonstrates that SUV can be used to quantify ^{68}Ga -PSMA-11 uptake in primary prostate cancer patients. A previously published study,⁽⁵³⁾ validating the kinetics of the prostate cancer tracer ^{18}F -Fluorocholine, showed that SUV could not be used to quantify ^{18}F -Fluorocholine uptake due to poor correlation between SUV and K_i , demonstrating the importance of undertaking quantitative studies to validate simplified semi-quantitative methods.

Interestingly, a comparison of the SUV between time-windows 15-30 min, 30-45min and 45-60min showed that the strongest correlation between K_i and SUV was not seen for the late time-window, which is the one usually studied in previous publications and recommended by published guidelines,^(34,60-62) but rather the 30-45min

time-window. The analysis of the optimal time-window for medical image reporting showed in agreement with the correlation analysis that the lesions close to the bladder were more easily outlined in early time frames than in late, mainly due to lower bladder activity in early time frames, without impairment of detectability. Earlier scanning should also provide a better image signal-to-noise ratio, due to less decay time, and finally it would result in a shorter total examination time for the patient. Future studies are needed to determine the optimal time-window for image reporting and quantification.

One of the main limitations of the study is the relatively small number of patients, although most published studies on pharmacokinetic modeling using arterial blood sampling have been done in small groups of patients due to the invasiveness of the method. We were however able to find significant differences between regions and time-windows with only 14 patients. The lack of correlation between PET and MR parameters might be due to the small patient sample. Another limitation of the study is the variation in injected activity, which in most cases was related to delay in the arterial puncture procedure or in the setup of the blood sampling device, resulting in decayed activity. For some patients, the data were indeed noisy, but it did not affect the pharmacokinetic analysis and SUV computations considerably.

5 CONCLUSIONS

1. The pharmacokinetics of ^{68}Ga -PSMA-11 can be described by an irreversible two-tissue compartment model. The rate constants K_1 , k_3 and K_i were significantly larger in lesion in comparison to muscle and normal prostate. ^{68}Ga -PSMA-11 is stable *in vivo*, which excludes the need for metabolite correction of plasma data;
2. Standardized uptake value correlated significantly with the net influx rate K_i , with the strongest correlation at 30-45 min. Standardized uptake value can therefore be used in clinical settings to quantify ^{68}Ga -PSMA-11 uptake;
3. The image-derived blood using a magnetic resonance -guided method agreed well with the arterial input function. The net influx rate K_i (Image-derived input function) showed excellent agreement with K_i (Arterial input function);
4. Standardized uptake value and K_i correlated significantly with serum prostate-specific antigen and prostate-specific antigen density, but not with multiparametric magnetic resonance parameters or histopathological data.

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Resumo

Introdução: A tomografia por emissão de pósitrons com o radiofármaco Glu-NH-CO-NH-Lys(Ahx)-HBED-CC marcado com ^{68}Ga (PSMA-11- ^{68}Ga), direcionada ao antígeno de membrana prostático específico tem demonstrado grande potencial para detecção e estadiamento do câncer de próstata. **Objetivos:** Quantificar a captação do PSMA-11- ^{68}Ga com modelagem farmacocinética e verificar métodos simplificados de quantificação. **Métodos:** Quinze pacientes com câncer de próstata foram submetidos à tomografia por emissão de pósitrons/ressonância magnética com PSMA-11- ^{68}Ga . Foram adquiridas imagens dinâmicas da região pélvica durante 60 minutos imediatamente após a administração do radiofármaco. Imagens de T1 Dixon, T2 e de difusão por ressonância magnética foram adquiridas simultaneamente. A atividade no sangue arterial foi medida com um contador automático e amostras de sangue arterial foram coletadas manualmente para corrigir a curva pela razão plasma/sangue total e análise metabólica. Curvas dinâmicas de volumes de interesse em lesões, próstata normal e músculo foram geradas e os valores padronizados de captação foram calculados. A farmacocinética do PSMA-11- ^{68}Ga foi avaliada por modelos compartimentais. Curvas de sangue derivadas da imagem utilizando um método baseado na ressonância magnética foram geradas e avaliadas como uma alternativa à coleta de sangue. As constantes do modelo cinético foram correlacionadas com valores padronizados de captação e com dados do paciente e da ressonância magnética. **Resultados:** Foram identificadas 18 lesões na tomografia por emissão de pósitrons/ressonância magnética. PSMA-11- ^{68}Ga mostrou-se estável *in vivo*, não requerendo correção por metabolismo. A cinética pode ser descrita por um modelo irreversível de dois compartimentos de tecido. As constantes cinéticas K_1 e k_3 e a constante de fluxo total de entrada K_i foram maiores nas lesões do que no tecido normal ($p < 0.05$). K_i calculada usando sangue arterial e sangue derivado da imagem mostraram excelente concordância com coeficiente de correlação intraclasse de 0.99. K_i mostrou uma correlação mais forte com valores padronizados de captação do tempo de exame 30-45min ($\rho = 0.95$, $p < 0.001$). K_i e os valores padronizados de captação mostraram correlação significativa com antígeno prostático específico e com a densidade do antígeno prostático específico. **Conclusões:** A cinética do PSMA-11- ^{68}Ga pode ser descrita por um modelo irreversível de dois compartimentos de tecido. A curva de sangue derivada da imagem pode ser utilizada como uma alternativa à coleta

de sangue. Os valores padronizados de captação apresentaram uma correlação significativa com K_i e podem ser usados na prática clínica para quantificar a captação do PSMA-11- ^{68}Ga .

Descritores: Neoplasias da próstata; Tomografia por emissão de pósitrons; Imagem por ressonância magnética; Farmacocinética

Appendices

Appendix 1. Equipment used in the study

Left: Automatic module for production and radiolabeling of ^{68}Ga -PSMA-11. Right: $^{68}\text{Ge}/^{68}\text{Ga}$ generator for ^{68}Ga elution.

Left: PET/MR scanning and simultaneous arterial blood sampling. Right: Automatic blood sampling device for online measurement of activity in arterial blood.

Arterial blood sampling setup using the twilite detector (Swisstrace)

Left: Single-sample well counter (HIDEX) for measurement of activity in whole blood and plasma.

Right: Centrifugation of manual blood samples to separate plasma.

UHPLC (Agilent) analysis and collection of aliquots for subsequent measurement in the gamma counter

Appendix 2. Image-derived input function

Segmentation of both external iliac arteries visible on Dixon MR image.

Co-registered vessel mask on PET image (red), where the VOI used to derive the IDIF is marked in green.

Appendix 3. Compartment model equations

Irreversible one-tissue compartment model (1T1k):

$$\frac{dC_1(t)}{dt} = K_1 C_P(t)$$

Reversible one-tissue compartment model (1T2k):

$$\frac{dC_1(t)}{dt} = K_1 C_P(t) - k_2 C_1(t)$$

Irreversible two-tissue compartment model (2T3k):

$$\begin{aligned}\frac{dC_1(t)}{dt} &= K_1 C_P(t) - k_2 C_1(t) - k_3 C_1(t) \\ \frac{dC_2(t)}{dt} &= k_3 C_1(t)\end{aligned}$$

Reversible two-tissue compartment model (2T4k):

$$\begin{aligned}\frac{dC_1(t)}{dt} &= K_1 C_P(t) - k_2 C_1(t) - k_3 C_1(t) + k_4 C_2(t) \\ \frac{dC_2(t)}{dt} &= k_3 C_1(t) - k_4 C_2(t)\end{aligned}$$

Total tissue concentration as a function of time:

$$C_T(t) = C_1(t) + C_2(t)$$

VOI activity, corrected for the fractional blood volume in tissue V_B :

$$C_{VOI}(t) = (1 - V_B)C_T(t) + V_B C_{WB}(t)$$