Quantitative tumor perfusion imaging with $^{82}$Rubidium-PET/CT in prostate cancer – analytical and clinical validation

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Word Count
5000

Abstract Word Count
287

Financial Support
This work was financially supported by The Danish Cancer Society, Health Research Fund of Central Denmark Region and Aage og Johanne Louis-Hansens Fond.
Short Running Title

$^{82}$Rb-PET/CT in prostate cancer
ABSTRACT
The aim of this work was to evaluate $^{82}$Rubidium ($^{82}$Rb) positron emission tomography (PET) / computed tomography (CT) as a diagnostic tool for quantitative tumor blood flow (TBF) imaging in prostate cancer (PCa). Study 1 was performed to evaluate $^{82}$Rb as a marker of TBF, using $^{15}$O-H$_2$O-PET as reference method. Study 2 investigated the ability of $^{82}$Rb uptake measurements to differentiate between PCa and normal prostate.

Methods

Study 1. Nine PCa patients scheduled for radical prostatectomy were included. Prostate multiparametric magnetic resonance imaging (mpMRI) and both cardiac and pelvic $^{15}$O-H$_2$O-PET and $^{82}$Rb-PET were performed. PET findings were compared to post-prostatectomy Gleason grade group (GGG).

Study 2. Fifteen primary high-risk PCa patients and 12 controls without known prostate disease were included in a clinical drug trial (EudraCT 2016-003185-26). $^{68}$Ga-prostate specific membrane antigen (PSMA)-PET/CT scans of PCa patients were available. Pelvic $^{82}$Rb-PET was performed.

Results

Study 1. Both $^{82}$Rb $K_1$ and $^{82}$Rb standard uptake values (SUV) correlated strongly with $^{15}$O-H$_2$O TBF ($\rho=0.95$, $p<0.001$ and $\rho=0.77$, $p=0.015$, respectively). $^{82}$Rb-SUV and $K_1$ were linearly correlated ($r=0.92$, $p=0.001$). $^{82}$Rb-SUV correlated with post-prostatectomy GGG ($\rho=0.70$, $p=0.03$).

Study 2. $^{82}$Rb-SUV in PCa ($3.19 \pm 0.48$) was significantly higher than prostate $^{82}$Rb-SUV in healthy controls ($1.68 \pm 0.37$) ($p<0.001$), with no overlap between groups.

Conclusion

Study 1 shows that $^{82}$Rb-PET/CT can be used for TBF quantification, and that TBF can be estimated by simple SUV; and suggests that $^{82}$Rb-SUV is associated with post-prostatectomy GGG and, hence, cancer aggressiveness. Study 2 shows that $^{82}$Rb-uptake is significantly higher in PCa than in normal prostate tissue with no overlap between cohorts, confirming the primary hypothesis of the clinical trial. Consequently, $^{82}$Rb-PET/CT may have potential as a non-invasive tool for evaluation of tumor aggressiveness and monitoring in non-metastatic PCa.

Key Words
$^{82}$Rubidium-PET, $^{15}$O-H$_2$O-PET, prostate cancer, tumor perfusion, cancer
INTRODUCTION

Prostate cancer (PCa) is the most common malignancy in men and responsible for 8% of male cancer deaths, making it a major cause of cancer deaths (1,2). However, the disease is heterogenous and the majority of patients with PCa will die from other causes, even with conservative cancer management. Consequently, the classification into significant cancer and insignificant cancer, which rely on risk evaluation, becomes an important challenge in PCa management (3).

The PCa diagnosis and risk evaluation is usually based on prostate specific antigen (PSA) sampling, digital rectal examination and random trans-rectal biopsies, which is both unpleasant and causing potentially severe side effects (4). PCa, including high-grade cancers, is common among men with PSA values under 4.0 ng/ml (5) and the number of false negative random sextant biopsies are relatively high (6). As this procedure only presents a rough assessment of the underlying tumor biology, it results in considerable overtreatment (7).

The use of multiparametric magnetic resonance imaging (mpMRI) using Prostate Imaging Reporting and Data System (PI-RADS) score (8), for diagnosis (9), biopsy guidance, and monitoring of PCa patients (10) have increased over the past years as mpMRI has a high sensitivity, specificity and negative predictive value (11). MpMRI has thereby made a major impact on detection of clinically significant PCa (3). Although an inverse relationship between apparent diffusion coefficient (ADC)-value and aggressiveness determined by Gleason grade has been established in peripheral zone cancers, the mpMRI does not assess the exact tumor aggressiveness. As induction of angiogenesis is one of the hallmarks of cancer (12,13), a non-invasive quantitative estimation of tumor blood flow (TBF) may assess tumor aggressiveness. This would enable repeated measurements of the tumors malignant potential, which could make a valuable contribution to the existing panel of diagnostic imaging in PCa at the time of diagnosis, but especially in evaluation of patients in active surveillance or active treatment.

TBF is generally increased in more aggressive PCa, and contrast-enhanced imaging provides additional information of functional tumor characteristics in mpMRI (14). Dynamic $^{15}$O-H$_2$O-positron emission tomography (PET) is the gold standard of measuring blood flow in humans. Recently, we demonstrated that absolute quantification values of perfusion measured by dynamic $^{15}$O-H$_2$O-PET in PCa are highly correlated with post-prostatectomy Gleason grade group (GGG) (15). Thus, this method is a promising non-invasive diagnostic tool for measurement of tumor aggressiveness. However, the method requires an on-site cyclotron to produce the short-lived $^{15}$O tracer, which is not possible in most clinical PET centers. Several other PET tracers are used for quantification of blood flow. A clinically interesting alternative
to $^{15}$O-H$_2$O is $^{82}$Rubidium ($^{82}$Rb), a potassium analogue with intracellular trapping in metabolically active tissues at a rate proportional to tissue blood flow. Contrary to $^{15}$O-H$_2$O, $^{82}$Rb is retained in the tissue allowing both absolute quantifications using kinetic modeling and semi-quantitative measurements using standard uptake values (SUV) in late uptake images. $^{82}$Rb is generator produced and is widely used for myocardial blood flow quantification in high throughput clinical settings. Consequently, $^{82}$Rb could be a relevant tracer for PCA blood flow measurements in a clinical setting, but has previously not been studied in this context.

Validating a tracer for a novel indication requires several steps and here we present two sub-studies. The main purposes of study 1 were to perform an analytical validation of tumor perfusion measurement by dynamic $^{82}$Rb-PET compared to the gold standard method dynamic $^{15}$O-H$_2$O-PET; to determine whether simplified semi-quantitative measurements using SUV can be used comparatively with absolute kinetic quantification; and finally, to evaluate the correlation between TBF and characteristics of PCA aggressiveness. The main purpose of study 2 was to compare TBF with blood flow in healthy prostate tissue in a control group without known prostate disease as the primary endpoint of a clinical trial.

MATERIALS AND METHODS

Patient Population

Study 1. Nine patients with PCa, scheduled for radical prostatectomy were included in a pilot study. Due to irregularity in the $^{82}$Rb infusion, one patient was excluded from kinetic analysis. mpMRI, PSA and post-prostatectomy GGG were available in these patients. As illustrated in Figure 1, four PET scans were performed per patient, pelvic and cardiac dynamic $^{15}$O-H$_2$O-PET and $^{82}$Rb-PET. PET images were fused to MRI, for tissue delineation for volume-of-interest (VOI) analysis.

Study 2. Fifteen patients with high-risk PCa (D’Amico criteria (16)) who had undergone $^{68}$Ga-prostate specific membrane antigen (PSMA)-PET/computed tomography (CT) were included. $^{68}$Ga-PSMA-PET/CT, PSA and needle biopsy GGG were available in these patients. Pelvic $^{82}$Rb-PET was performed (Fig. 1). $^{82}$Rb-PET images were fused to $^{68}$Ga-PSMA-PET/CT for tissue delineation for VOI analysis. Fifteen controls were recruited from patients referred for myocardial blood flow examination with $^{82}$Rb-PET. The controls had no urinary tract symptoms, no known prostate disease
and a PSA blood sample was taken. Three controls were subsequently excluded. Pelvic $^{82}$Rb-PET/CT scan was performed before clinical cardiac examination.

The institutional review board (Central Denmark Region Committees on Health Research Ethics) approved both studies and all subjects signed a written informed consent. The Danish Medicines Agency approved study 2 as a drug trial monitored by the Unit of Good Clinical Practice, EudraCT number 2016-003185-26.

**Imaging**

*Study 1.* Both pelvic and cardiac $^{15}$O-H$_2$O-PET scans were performed on a Siemens Biograph Truepoint PET/CT scanner (Siemens, Erlangen, Germany). Bolus injection of $^{15}$O-H$_2$O (400 MBq) at the beginning of each scan was performed with a MedRad Contrast Infusion Pump (1 ml/s) followed by infusion of 30 mL saline. Arterial blood sampling was performed using an automatic blood sampler (Allogg ABSS, Mariefred, Sweden) and delay and dispersion correction was performed. Both pelvic and cardiac $^{82}$Rb-PET scans of the patients were performed on a GE Discovery 690 PET/CT scanner (GE Healthcare, Waukesha, Wisconsin, USA). A low-dose CT scan was performed prior to the scans for attenuation correction. $^{15}$O-H$_2$O and MRI scans have previously been described in Tolbod et al. (15).

*Study 2.* Pelvic $^{82}$Rb scans of the patients and controls were performed on a GE Discovery MI Digital Ready PET/CT (GE Healthcare, Waukesha, Wisconsin, USA). $^{68}$Ga-PSMA-PET/CT was performed according to clinical guidelines.

*Study 1 and 2.* Both GE scanners have the same PET-detector configuration and the same PET reconstruction algorithm was used. A low-dose CT was used for attenuation correction and all common corrections were applied. Scans (8 min) were performed in list mode with extraction of a static (3 to 7 min post injection) and a dynamic image series (frame structure: 22x5s, 6x10s, 4x20s, 4x40s, 1x60). PET images were reconstructed using the VuePointFX reconstruction algorithm (2 iterations 24 subsets) in a 3.27x3.27x3.27 mm (5 mm transaxial Gaussian postfilter and 3-point axial convolution postfilter [1 4 1]) and a 2.18x2.18x3.27 mm matrix (4 mm transaxial Gaussian postfilter and 3-point axial convolution postfilter [1 61]) for the static and dynamic series, respectively. Bolus injection of $^{82}$RbCl (1110 MBq) at the beginning of each scan was performed directly by the Cardiogen-82 generator infusion system (Bracco, Monroe Township, New Jersey, USA).
Image Analysis

Study 1. The T2 weighted mpMRI images were fused with the low-dose CT scans and, subsequently, both the $^{15}$O-$\text{H}_2$O and $^{82}$Rb-PET scans using Carimas software (Turku PET-Centre, Finland) (17). The tumor VOI were drawn directly on the mpMRI, using both T2 weighted and diffusion weighted images. The VOIs were transferred to the PET series and time-activity curves were extracted. To calculate $K_1$, input function from arterial blood sampling was used for $^{15}$O-$\text{H}_2$O PET and cardiac image derived arterial input function was used for $^{82}$Rb PET, as described in details by Tolbod et al. (15). Kinetic modelling using a 1-tissue compartment model for both tracers was performed (15). SUV analysis was performed using the static image series (3 to 7 min post injection).

Study 2. The $^{68}$Ga-PSMA-PET/CT scans were fused with the low-dose CT scans and the $^{82}$Rb-PET scans using Hybrid Viewer (Hermes Medical Solutions, Stockholm, Sweden). The tumor VOI were drawn directly on the $^{68}$Ga-PSMA-PET/CT images by visual guidance from PSMA activity and transferred to the $^{82}$Rb-PET static images (primary endpoint) and by using 60\% threshold 3D-VOI at the $^{82}$Rb-PET hot spot (secondary endpoint). The VOI of the total prostate gland were drawn manually on the low-dose CT scan of both patients and controls. Furthermore, VOIs of the bladder, seminal vesicles and bone were drawn for obtaining normal tissue reference values. No kinetic analysis was performed.

Statistical Analysis

Study 1. Correlation between tumor perfusion measured by $^{15}$O-$\text{H}_2$O $K_1$ and $^{82}$Rb-PET $K_1$ and between $^{15}$O-$\text{H}_2$O $K_1$ and $^{82}$Rb-PET SUV were analyzed using Spearman’s rank correlation, as the correlation is monotonic but non-linear. The linear correlation between $^{82}$Rb-PET $K_1$ and $^{82}$Rb-SUV were analyzed using Pearson’s correlation.

Study 2. The difference in tumor perfusion drawn by visual PSMA guidance and by 60\% threshold 3D-VOI method of the patients compared to normal prostate tissue in the controls were analyzed using T-test for difference in means.

Study 1 and 2. Correlations between tumor $^{82}$Rb-SUV and PSA was analyzed using Pearson’s correlation. Correlations between tumor $^{82}$Rb-SUV and GGG were analyzed using Spearman’s rank correlation, as GGG is an ordinal scale. Data was tested for normality using Shapiro-Wilk W test. P-values < 0.05 were considered statistically significant. Analysis was performed in STATA version 15.1 (StataCorp LLC, College Station, Texas, USA).
RESULTS

Dynamic \(^{82}\text{Rb}\)-PET/CT and \(^{82}\text{Rb}\)-SUV Measure Prostate TBF Precisely

In study 1 TBF estimated from \(^{15}\text{O-H}_{2}\text{O}\) \(K_1\)-images and \(^{82}\text{Rb}\)-PET \(K_1\)-images were highly correlated, as shown in Figure 2 (rho=0.95, p<0.001). The correlation between \(^{15}\text{O-H}_{2}\text{O}\) \(K_1\) and \(^{82}\text{Rb}\)-PET SUV\text{mean} was (rho=0.77, p=0.015). The curve levels off at values above 0.3 mL/min/mL in full accordance with the known non-linear correction for incomplete extraction of \(^{82}\text{Rb}\) known from myocardial blood flow measurements (18). Thus, our data matches the incomplete extraction curve of \(^{82}\text{Rb}\) quite well.

Figure 3 illustrates an excellent correlation between \(^{82}\text{Rb}\) blood flow measurement with \(K_1\)-images using cardiac image derived input functions and \(^{82}\text{Rb}\)-SUV\text{mean} (r=0.92, p=0.001). An example of the series of images in study 1 can be found in Figure 4A (patient 3).

Blood Flow in Prostate Cancer is Higher than in Healthy Prostate Tissue

Figure 4B shows examples of \(^{82}\text{Rb}\)-scans of the controls of study 2, ranging from the lowest SUV\text{mean} (left) to the highest SUV\text{mean} (right). For comparison, two examples of the correlation between \(^{68}\text{Ga-PSMA-PET}\) and \(^{82}\text{Rb}\)-PET are shown in Figure 4C. Both tumors had highly increased blood flow on \(^{82}\text{Rb}\)-PET images.

Tumor \(^{82}\text{Rb}\)-SUV\text{mean} of the patients, drawn by PSMA guidance 3.19 [2.91 - 3.46] was significantly higher (p<0.001) than \(^{82}\text{Rb}\)-SUV\text{mean} in healthy prostate tissue of the controls 1.68 [1.44 - 1.91] (Fig. 5) (primary endpoint). The same was found for \(^{82}\text{Rb}\)-SUV\text{mean} estimated without external guidance using the 60% threshold method on \(^{82}\text{Rb}\)-PET hot spot 3.85 [3.39 - 4.30] (secondary endpoint).

Association Between TBF and Characteristics of Tumor Aggressiveness

Study 1. The TBF correlated with post-prostatectomy GGG (rho=0.70, p=0.03) (Fig. 6) and PSA (r=0.88, p=0.002). Additional patient characteristics are found in Table 1.

Study 2. No significant correlation was found between \(^{82}\text{Rb}\)-SUV and random biopsy GGG (rho=0.21, p=0.46) or between \(^{82}\text{Rb}\)-SUV and PSA (r=0.20, p=0.47). The patient characteristics are found in Table 2.
Blood flow in Prostate Cancer Metastases and in Normal Tissues

Two patients in study 2 (9 and 15) had bone metastases in the field-of-view, SUVmean of these were 2.40, which was significantly higher than normal bone tissue (p=0.005). One patient (Patient 9) had a large lymph node metastasis in the field-of-view with SUVmean of 2.61, which was markedly increased compared to soft tissue in general.

Normal tissue reference values drawn from the healthy controls of the bladder 0.57 [0.44 – 0.70], seminal vesicles 1.07 [0.89 – 1.24] and bone 1.12 [0.92 – 1.32] are found in Table 3 along additional control characteristics.

Detection of Bilateral High-risk Prostate Cancer with $^{82}$Rb-PET/CT

A 69-year-old man, recruited as control subject in study 2, without known prostate disease, no lower urinary tract symptoms and normal PSA 3.1 ng/ml. The $^{82}$Rb-PET/CT showed a left-side peripheral zone focus with intense $^{82}$Rb-uptake (Fig. 7A) and a small right-side peripheral zone focus with increased $^{82}$Rb-uptake (Fig. 7B). Because of the suspicious prostate lesions on $^{82}$Rb-PET/CT, the patient was referred for prostate MRI. The MRI revealed a correlating left-side 7x9x11mm PI-RADS 4 peripheral zone lesion (Fig. 7C) with ADC-value 580·10^{-6} mm²/s (Fig. 7D) and a right-side 7x7x7mm PI-RADS 4 peripheral zone lesion (Fig. 7C) with ADC-value 774·10^{-6} mm²/s (Fig. 7D). In-bore MRI-guided biopsies were taken (Figs. 7E and 7F), revealing Gleason 4+5 in 67% and 42 % of needle length from the left and right lesion, respectively.

A total of three controls were excluded; one was referred for urological examination due to elevated PSA-value (exclusion criteria); one due to bilateral metallic hip implants, causing massive scan artefacts; and finally, the one described above, due to high-risk PCa (exclusion criteria).

DISCUSSION

The main results of study 1 show that TBF estimated from $^{15}$O-H₂O K₁, $^{82}$Rb-PET K₁ and $^{82}$Rb-PET SUV are highly correlated; and that $^{82}$Rb-SUV is associated with post-prostatectomy GGG and, hence, cancer aggressiveness. Furthermore, study 2 showed that $^{82}$Rb uptake is higher in PCa than in normal prostate tissue.
Dynamic $^{82}\text{Rb}$-PET/CT and $^{82}\text{Rb}$-SUV Measure Prostate TBF Precisely

Blood flow is an underlying basis for tumor growth (12,13). As perfusion is defined by trans-capillary flux of water, which can be measured and quantified by $^{15}\text{O-H}_2\text{O}$-PET/CT, this is considered the gold standard for non-invasive measurement of blood flow in humans. $^{15}\text{O-H}_2\text{O}$-PET perfusion in PCa patients is significantly higher than in normal prostate tissue and benign prostate hyperplasia (19). Absolute quantification of perfusion was demonstrated to correlate well with PCa aggressiveness (15), and could hence, be a valuable tool in risk evaluation and monitoring of PCa. However, $^{15}\text{O-H}_2\text{O}$-PET /CT remains a challenging imaging modality due to the requirement of an on-site cyclotron to produce the short-lived $^{15}\text{O}$ tracer, which is not possible in most clinical PET centers.

The present pilot study (study 1) validates $^{82}\text{Rb}$-PET/CT as a diagnostic tool for quantitative measurement of TBF by demonstrating that TBF estimates from $^{15}\text{O-H}_2\text{O}$-PET and $^{82}\text{Rb}$-PET $K_1$ images were highly correlated. The correlation between $^{82}\text{Rb}$-PET and $^{15}\text{O-H}_2\text{O}$-PET is not linear as the fit line flattens at high perfusion values, causing $^{82}\text{Rb}$-PET to underestimate the high perfusion areas. This was expected as the same relation between $^{82}\text{Rb}$-PET and $^{15}\text{O-H}_2\text{O}$-PET is known from cardiac $^{82}\text{Rb}$-PET imaging and caused by the incomplete $^{82}\text{Rb}$ extraction (18).

A simple image analysis for retention tracers is the use of semi-quantitative SUV values as a substitute for TBF. Since $^{82}\text{Rb}$-PET $K_1$ and $^{82}\text{Rb}$-SUV were highly correlated in our study, this approach seems applicable, and it simplifies the image reconstruction and scan analysis. Since the tumor outline was known in all patients, SUVmean was selected instead of SUVmax to reduce noise bias.

Blood Flow in Prostate Cancer is Higher than in Healthy Prostate Tissue

In study 2 TBF estimated by $^{82}\text{Rb}$-PET SUV is significantly higher than SUVmean of presumed healthy prostate tissue of the controls with no overlap between the groups. However, the tumor with lowest TBF and the control with highest mean prostate blood flow are not far from each other. This is explained by areas of transitional zone benign hyperplasia in some of the healthy controls. These areas display high blood flow on $^{82}\text{Rb}$-PET, whereas the healthy peripheral zone generally has homogenous and low blood flow (Fig. 4B). Thus, the tumor-background contrast for peripheral zone tumors is better than would seem from the statistics. This was also illustrated by the case of the control that was diagnosed with PCa on $^{82}\text{Rb}$-PET (Fig. 7). On the other hand, transitional zone tumors can be challenging to differentiate from benign hyperplasia nodules on $^{82}\text{Rb}$-PET alone. However, with MRI guidance or PSMA-PET/CT
guidance, the tumor can be outlined. Hence, it is crucial to be familiar with prostate anatomy, including usual localization of PCa and hyperplasia for interpretation of prostate $^{82}\text{Rb}$-PET.

Our finding that tumor $^{82}\text{Rb}$-PET SUV is significantly higher than SUVmean of the prostate gland of healthy controls is consistent with previous SPECT studies with the radioisotope Thallium-201, which was able to differentiate cancer from benign prostate hyperplasia (20, 21). The uptake of Thallium-201 in tumor cells is mediated by the Na-K ATPase and the Na-K-2Cl cotransporter (22), and hence the biological properties of Thallium-201 are similar to those of $^{82}\text{Rb}$.

**Association Between TBF and Characteristics of Tumor Aggressiveness**

In study 1 TBF measured by $^{82}\text{Rb}$-PET correlated with post-prostatectomy GGG. Post-prostatectomy histopathological evaluation with Gleason Grading is the best estimate of aggressiveness and cancer growth. Since TBF illustrates the metabolic needs of the tumor, this correlation is explainable. However, there was no correlation between TBF measured by $^{82}\text{Rb}$-PET and GGG from random prostate biopsies in study 2. This might be explained by the fact that random biopsy Gleason score often differs from post-prostatectomy Gleason score and thus GGG (23). This might be an indication that $^{82}\text{Rb}$-PET SUV is superior to random biopsies for preoperative risk assessment. However, this needs to be evaluated further in large clinical studies.

The results of study 1 and study 2 differed regarding the correlation between TBF and PSA. An excellent correlation was found in study 1 and no correlation was found in study 2. Such a correlation might be rooted in the same logic as that which explained the correlation with GGG; namely that the amount of PSA produced in the tumor is related to the metabolism, as illustrated by the TBF. In study 2, the two patients with highest PSA values (patient 9 and 15) both had multiple metastases and quite low blood flow in the primary prostate tumor. This might explain the lack of correlation between TBF and PSA in study 2 and demonstrate that blood flow in the primary prostate tumor alone does not correlate with PSA in metastatic prostate cancer.

**Future Perspectives**

More studies are needed to determine the exact correlation between $^{82}\text{Rb}$-PET and PCa aggressiveness characteristics and about the predictive value of $^{82}\text{Rb}$-PET regarding long term clinical course of the disease. Finally, further
studies are needed to determine the characteristics and underlying biology of $^{82}$Rb-uptake in PCa, including the indicated connection to restricted diffusion on mpMRI.

If the demonstrated correlation between quantitative $^{82}$Rb-PET TBF and post-prostatectomy GGG, and hence aggressiveness, is consistent in larger studies, this method may be a valuable addition to the existing risk evaluation algorithm. $^{82}$Rb-PET can be merged with mpMRI, and perhaps even performed in PET/MRI scanners at some departments. Even though we managed to detect and diagnose the first patient with PCa on $^{82}$Rb-PET/CT, the method is not suited for initial detection of PCa, but only for characterization of localized tumors. MRI is obviously a superior modality for detecting and assessing the tumor morphology and anatomy, but if quantitative measurement of TBF is further proven to be reliable for non-invasive assessment of aggressiveness, there could be powerful synergy between the modalities. It may be interesting to investigate whether this modality can be used in the assessment of which patients are suited for active surveillance, and for monitoring patients under active surveillance. It could also be interesting to investigate the TBF response to therapy, and to evaluate whether the initial response is predictive of long-term treatment effect.

Since $^{82}$Rb uptake is associated with flow and aggressiveness in PCa, it may be relevant also to investigate the role of $^{82}$Rb uptake in other cancers.

CONCLUSION

Study 1 shows that $^{82}$Rb-PET/CT is a diagnostic tool for quantitative TBF imaging by validation against the gold standard method $^{15}$O-H$_2$O-PET /CT; that TBF can be estimated by $^{82}$Rb-PET/CT using simple SUV; and suggests that $^{82}$Rb-SUV is associated with post-prostatectomy GGG and, hence, cancer aggressiveness. Study 2 shows that $^{82}$Rb uptake is higher in PCa than in normal prostate tissue with no overlap between cohorts, confirming the clinical trials primary hypothesis. Consequently, $^{82}$Rb-PET/CT may have potential as a non-invasive tool for evaluation of tumor aggressiveness and monitoring in non-metastatic PCa.

DISCLOSURE

This work was financially supported by The Danish Cancer Society, Health Research Fund of Central Denmark Region and Aage og Johanne Louis-Hansens Fond. The authors declare no potential conflicts of interest.
REFERENCES


### TABLE 1: Patient characteristics from study 1. * Value missing due to irregularity in $^{82}$Rb infusion.

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<td>65</td>
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<td>0.22</td>
<td>0.29</td>
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<td>0.18</td>
<td>0.08</td>
<td>0.14</td>
<td>0.54</td>
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<td>TBF $K_1$-$^{82}$Rb (ml/min/ml)</td>
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<td>*</td>
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<td>TBF 60% threshold ($^{82}$Rb-SUVmean)</td>
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<td>4.11</td>
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**TABLE 2**: Patient characteristics from study 2. *Value missing due to very low/none PSMA uptake in the prostate gland.

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<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>68.13±5.97</td>
</tr>
<tr>
<td>Prostate (82Rb-SUVmean)</td>
<td>2.59</td>
<td>1.94</td>
<td>2.25</td>
<td>2.33</td>
<td>3.26</td>
<td>3</td>
<td>2.51</td>
<td>2.19</td>
<td>1.54</td>
<td>2.25</td>
<td>2.01</td>
<td>2.6</td>
<td>2.98</td>
<td>2.44</td>
<td>2.61</td>
<td>2.43±0.44</td>
</tr>
<tr>
<td>TBF PSMA guided (82Rb-SUVmean)</td>
<td>3.39</td>
<td>3.19</td>
<td>3.82</td>
<td>3</td>
<td>3.84</td>
<td>3.73</td>
<td>*</td>
<td>3.33</td>
<td>2.21</td>
<td>2.78</td>
<td>2.47</td>
<td>3.37</td>
<td>3.16</td>
<td>3.34</td>
<td>2.99</td>
<td>3.19±0.48</td>
</tr>
<tr>
<td>TBF 60% threshold (SUVmean)</td>
<td>5.78</td>
<td>2.99</td>
<td>3.93</td>
<td>3.57</td>
<td>4.91</td>
<td>4.31</td>
<td>3.96</td>
<td>3.29</td>
<td>2.4</td>
<td>3.33</td>
<td>3.8</td>
<td>3.52</td>
<td>3.84</td>
<td>3.51</td>
<td>4.55</td>
<td>3.85±0.82</td>
</tr>
<tr>
<td>Gleason Grade Group (Biopsy)</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>3.53±1.55</td>
<td></td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td>64.6</td>
<td>15.5</td>
<td>23.1</td>
<td>10.6</td>
<td>76.7</td>
<td>14.5</td>
<td>6.8</td>
<td>15.5</td>
<td>121.9</td>
<td>13.2</td>
<td>20.2</td>
<td>40.5</td>
<td>28.7</td>
<td>7.2</td>
<td>168.6</td>
<td>41.84±47.5</td>
</tr>
<tr>
<td>Prostate volume (CT) (cm³)</td>
<td>89.8</td>
<td>71.6</td>
<td>137.0</td>
<td>113.8</td>
<td>91.1</td>
<td>63.6</td>
<td>82.4</td>
<td>74.5</td>
<td>40.4</td>
<td>42.6</td>
<td>65.9</td>
<td>49.3</td>
<td>128.4</td>
<td>53.8</td>
<td>113.1</td>
<td>81.2±30.7</td>
</tr>
</tbody>
</table>
**TABLE 3:** Control characteristics. *Control 7 had notable higher urinary bladder SUVmean than the other controls due to increased renal excretion of potassium and $^{82}$Rb resulting in severe hypokalemia. Therefore, the value was left out of “normal tissue mean value**” regarding urinary bladder activity.

<table>
<thead>
<tr>
<th>Control number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>62.67±7.33</td>
</tr>
<tr>
<td>Prostate (SUVmean)</td>
<td>1.68</td>
<td>1.99</td>
<td>1.48</td>
<td>1.48</td>
<td>0.78</td>
<td>1.88</td>
<td>1.31</td>
<td>1.94</td>
<td>2.08</td>
<td>1.66</td>
<td>1.91</td>
<td>1.95</td>
<td>1.68±0.37</td>
</tr>
<tr>
<td>Seminal vesicles (SUVmean)</td>
<td>1.1</td>
<td>0.99</td>
<td>0.77</td>
<td>1.04</td>
<td>0.36</td>
<td>1.92</td>
<td>0.83</td>
<td>1.12</td>
<td>1.52</td>
<td>1.24</td>
<td>1.14</td>
<td>0.82</td>
<td>1.07±0.41</td>
</tr>
<tr>
<td>Bladder (SUVmean)</td>
<td>0.36</td>
<td>0.54</td>
<td>0.85</td>
<td>0.25</td>
<td>0.65</td>
<td>0.56</td>
<td>3.8*</td>
<td>0.56</td>
<td>0.58</td>
<td>0.89</td>
<td>0.62</td>
<td>0.37</td>
<td>0.57±0.19**</td>
</tr>
<tr>
<td>Bone (SUVmean)</td>
<td>0.7</td>
<td>0.91</td>
<td>0.75</td>
<td>0.97</td>
<td>0.96</td>
<td>1.63</td>
<td>1.45</td>
<td>1.67</td>
<td>1.23</td>
<td>1.1</td>
<td>1.05</td>
<td>1.01</td>
<td>1.12±0.32</td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td>2.9</td>
<td>0.6</td>
<td>1.4</td>
<td>1.9</td>
<td>0.8</td>
<td>0.6</td>
<td>0.5</td>
<td>0.7</td>
<td>1.2</td>
<td>1.1</td>
<td>0.9</td>
<td>0.4</td>
<td>1.08±0.71</td>
</tr>
<tr>
<td>Prostate volume (CT) (cm³)</td>
<td>79.9</td>
<td>83.5</td>
<td>61.8</td>
<td>61.2</td>
<td>30.5</td>
<td>41.7</td>
<td>36.0</td>
<td>37.8</td>
<td>88.9</td>
<td>59.8</td>
<td>57.5</td>
<td>46.5</td>
<td>57.1±19.4</td>
</tr>
</tbody>
</table>
**FIGURE 1:** Overview over study groups.

<table>
<thead>
<tr>
<th>Study 1</th>
<th>PCa patients</th>
<th>( n = 9 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>mpMRI + ( ^{15} \text{O-H}_2\text{O PET} + ^{82} \text{Rb PET} ) (prostate)</td>
<td>cardiac + pelvic</td>
<td>cardiac + pelvic</td>
</tr>
<tr>
<td></td>
<td>Prostatectomy + Histopathology</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study 2</th>
<th>PCa patients</th>
<th>( n = 15 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ^{68} \text{Ga-PSMA PET} + ^{82} \text{Rb PET} ) (whole body)</td>
<td>pelvic</td>
<td>pelvic</td>
</tr>
<tr>
<td></td>
<td>Biopsies + Histopathology</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study 2</th>
<th>Controls</th>
<th>( n = 12 )</th>
</tr>
</thead>
</table>
| \( ^{82} \text{Rb PET} \) (pelvic) | }
FIGURE 2: Rubidium vs water (tumor tissue only). Rho=0.95, p=0.001. Curve is extraction curve from Lortie et al (18).
FIGURE 3: Rubidium. SUV vs $K_1$ (All tissues). Tumor is shown in red, correlation for tumor only ($r=0.92$, $p=0.001$).
**FIGURE 4:** Row A shows patient 3 from study 1, from the left: T2w MRI, $^{15}$O-H$_2$O-PET K1 (80% of max), $^{82}$Rb-PET K1 (80% of max) and $^{82}$Rb-PET SUV. Row B shows $^{82}$Rb-PET of four controls from study 2, from the left: control 5 (lowest SUV mean), control 3, control 6 and control 9 (highest SUV mean). Row C shows $^{68}$Ga-PSMA-PET images and $^{82}$Rb-PET images of two patients from study 2. To the left a peripheral zone tumor (patient 14) and to the right a transitional zone tumor (patient 2).
FIGURE 5: Tumor Rubidium SUVmean of the patients in study 2 drawn by PSMA guidance compared to SUVmean of the entire prostate gland of the healthy controls.
**FIGURE 6:** Rubidium $SUV_{\text{mean}}$ vs post-prostatectomy GGG ($\rho=0.70$, $p=0.03$).
FIGURE 7: $^{82}$Rb-PET images (A and B) and MRI images of the control that turned out to have bilateral high-risk PCa. T2 weighted MRI (C), ADC-map (D) and axial and sagittal MRI TRUFI-sequence of the left-side biopsy (E and F). Yellow arrows indicate the left-side tumor, whereas blue arrows indicate the right-side tumor.