Journal of Nuclear Medicine, published on June 9, 2022 as doi:10.2967/jnumed.122.264101

The Impact of Peptide Amount on Tumor Uptake to Assess PSMA Receptor Saturation on ^{68}Ga-PSMA-11 PET/CT in Primary Prostate Cancer Patients

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Word count: 4585

Short running title: PSMA saturation after ^{68}Ga-PSMA-11

ABSTRACT

Rationale Gallium-68 (^{68}Ga)-labelled Prostate-specific Membrane Antigen (PSMA) is often produced on-site, where usually a fixed amount of peptide is conjugated to the generator-eluate. However, fluctuations in specific activity might influence tracer distribution and tumor accumulation. Therefore, our aim was to investigate the potential effect of varying administered peptide amount on ^{68}Ga-PSMA-11 uptake in tumor lesions using PET/CT in patients with primary prostate cancer (PCa). Additionally, the impact of tumor lesion volume on this potential effect and on accumulation in reference organs was assessed.

Methods Imaging data of 362 men with primary PCa who received ^{68}Ga-PSMA-11 PET/CT were retrospectively included. Scan quantification was performed for normal tissue and primary tumor lesions. Patients were divided into three groups based on their tumor lesion volume. Correlation and (multivariable) linear regression analyses were performed.

Results Median index lesion volume was 9.50 mL (range 0.064–174 mL). Groups were based on quartiles of prostatic lesion volume: ≤4.11 mL (group 1), 4.11–20.6 mL (group 2), and ≥20.6 mL (group 3). No correlation was found between administered peptide amount and tumor uptake (SUV_{peak} or SUV_{mean}) for all groups, except for a significant correlation for SUV_{mean} in the first group (p=0.008). Linear regression analysis supported these findings.

Conclusion No evident effect was observed of administered peptide amount on ^{68}Ga-PSMA-11 uptake in tumor lesions, except for a significant positive correlation between administered peptide amount and tumor SUV_{mean} for group 1. Findings imply that no receptor saturation occurs in men with primary PCa at peptide levels of ~2.5 µg**.**

Keywords: PSMA, prostate cancer, peptide amount, receptor saturation, specific activity

INTRODUCTION

Prostate-specific Membrane Antigen (PSMA) ligands target the PSMA receptor, which is significantly overexpressed on the surface of prostate cancer (PCa) cells (*1*). Radiolabeled PSMA-directed ligands are increasingly used for both diagnosis and therapy in PSMA positive PCa. The first clinical PSMA-directed tracer, Gallium-68 (^{68}Ga)-labelled PSMA, is a highly tumor-specific biomarker that is used for diagnosis and staging of both primary and (oligo)metastatic PCa nowadays (*2-4*). The commercial availability of Germanium-68 (^{68}Ge)/^{68}Ga-generators and PSMA-ligands has ensured that this development is now firmly embedded in many clinics.

The on-site labeling procedure of ^{68}Ga-PSMA-11 involves conjugation of a usually fixed amount of peptide to the generator-eluate, since total vials that hold a fixed amount PSMA-peptide are generally used during labeling procedures. The administered amount of radioactivity is often standardized between patients, to ensure a comparable inter- and intrapatient image quality. However, radioactivity levels in the ^{68}Ge/^{68}Ga-generator decrease over its lifetime, thus resulting in variable elution efficiencies, while the amount of peptide added to this generator-eluate is kept constant. Because patients receive approximately equal radioactivity doses, total peptide amounts per injection will vary. In receptor-based imaging and therapy, inconstant specific activities can lead to altered tumor accumulation profiles due to varying levels of receptor occupancy in target and non-target tissues. Though this effect has been demonstrated before in pharmacokinetic models for therapeutic doses Yttrium-90 labeled DOTATATE (*5*), and in clinical cases for Zirconium-89 labelled antibodies (*6*), it has not yet been demonstrated for ^{68}Ga-PSMA-11.

Previously, our group showed variable ^{68}Ga-PSMA-11 uptake profiles in primary PCa lesions using dynamic PET/CT in a small population of men with localized PCa (*7*). It was hypothesized that this might be explained by different receptor saturation states of intraprostatic PCa lesions. After all, an uptake plateau could correspond to a small tumor having a limited total receptor amount, and thus reaching total occupancy of available receptors, while such a total occupancy of receptors will probably not occur in larger tumors or (oligo)metastatic PCa that tend to have a larger amount of PSMA receptors. Presence of tumor receptor

saturation may potentially be relevant in PSMA-based radionuclide therapy, as usually much larger peptide amounts are administered (e.g. ~250 µg using lutetium-177 (^{177}Lu)-PSMA), compared to imaging with ^{68}Ga-PSMA-11 (~5 µg). In addition, interpatient variability in administered ^{68}Ga-PSMA-11 peptide amounts could lead to differences in tumor and organ distribution if receptor saturation would take place.

For this reason, there is an urgent need to define whether or not receptor saturation occurs in ^{68}Ga-PSMA-11 distribution in primary PCa and if this is dependent on lesion volume. Our research aims to assess these uncertainties were 1) to quantitatively investigate the potential effect of varying administered peptide amount on ^{68}Ga-PSMA-11 uptake in tumor lesions using PET/CT in patients with primary PCa and 2) to assess the impact of tumor lesion volume on this potential effect and on accumulation in reference tissue.

MATERIALS AND METHODS

Study Population

This retrospective data analysis study was approved by the Institutional Review Board (IRBd20-201) of the Netherlands Cancer Institute (Amsterdam, The Netherlands). All patients had given informed consent for use of the clinically obtained data for routine care via institutional procedures. Data of men with intermediate/high-risk (either ≥cT3, Gleason score ≥ 7 or prostate-specific antigen ≥ 20 ng/mL) primary PCa who underwent ^{68}Ga-PSMA-11 PET/CT prior to treatment between January 2016 and May 2020 were included in this study. Patients were excluded from analysis if no PSMA positive lesion was visualized on ^{68}Ga-PSMA-11 PET/CT (defined as no intraprostatic accumulation higher than prostate background). Patients did not receive any hormone therapy prior to their scan.

Image Acquisition and Analysis

^{68}Ga-Glu-urea-Lys(Ahx)-HBED-CC (^{68}Ga-PSMA-11) was produced in-house using a fully automated system (Scintomics GmbH, Fürstenfeldbruck, Germany) by combining 10 µg PSMA-11 to the generator eluate. Patients were prepared and images acquired according to standard clinical protocols. An intravenous bolus of ~100 MBq (2016 until September 2019) or ~150 MBq (from September 2019 onward) ^{68}Ga-PSMA-11 was injected approximately 60 minutes before start of the scan. Whole-body (midthigh to skull base) scans were acquired on either a Gemini TF, Gemini TF BigBore or Vereos digital PET/CT system (Philips, Best, the Netherlands) using harmonized scan and reconstruction protocols (4x4mm voxel sizes). In addition, a low dose CT scan was acquired for attenuation correction and anatomical correlation.

Quantitative evaluations of imaging data were performed using 3D Slicer (slicer.org, version 4.11) (*8*). Since there is no gold standard for prostate tumor segmentation on PSMA-PET, two segmentation methods (i.e., standardized manual versus threshold based) were compared. Based on these results provided in the Supplemental Data, further analysis was performed by standardized manual segmentation of all lesions on PET/CT. Mean absolute uptake (MBq/mL), SUV_{mean} and SUV_{peak} were used to express ^{68}Ga-

PSMA-11 uptake. SUV_{peak} was defined as the 1 cm^3 that showed the highest activity concentration within the volumes-of-interest.

In all scans, spherical volumes-of-interest of ø2.0 cm were drawn to obtain normal tissue uptake in parotid, aortic arch, liver (ø5.0 cm), kidney cortex, and gluteal muscle. Normal tissue SUV_{mean} and SUV_{peak} values were normalized to the uptake in the aortic arch (referred to as 'blood pool'), and these ratios were then compared between patient groups. In case of multifocal intraprostatic disease, both lesions were segmented, but the most profound or largest lesion (referred to as 'index lesion') was used for initial analysis.

Statistical Analyses

Statistical analyses were performed in R (version 3.6.3) (*9*). Patients were divided into three groups based on index lesion volume quartiles (group 1: ≤Q1, group 2: Q1-Q3 and group 3: ≥Q3). Differences in patient characteristics between these groups were evaluated using an ANOVA test, or a Kruskal-Wallis test in case of non-normal distribution with Bonferroni corrections to account for multiple testing. Spearman's correlation coefficient tests were performed to investigate potential associations between administered PSMA peptide amount and observed organ and tumor uptake on PET per group. A linear regression analysis was performed to assess a potential interaction effect between administered peptide amount and index lesion volume on ^{68}Ga-PSMA-11 tumor uptake. In addition, a multivariable regression analysis was performed to identify other potential variables that impact ^{68}Ga-PSMA-11 tumor uptake, and thus, could complicate interpretation and comparison of the found results between groups. Parameters that were tested as covariates were: age, body mass index (BMI), estimated glomerular filtration rate (eGFR), injection-acquisition interval, scanner type, furosemide administration during scan, risk of PCa recurrence and disease spread. Risk of PCa recurrence was based on the D'Amiko risk classification (*10*). The disease spread, and thus also presence or absence of metastases, was categorized into four groups: local, locally advanced, oligometastatic and metastatic. The definition of locally advanced was staging with either T3 or T4, or N1 independent of T (*11*). Oligometastatic disease was defined as ≤5 metastatic tumor lesions

excluding ≤4 synchronous pelvic lymph nodes (*12*, *13*). A p-value less than 0.05 was considered statistically significant.

RESULTS

Initially, 391 patients were retrieved for inclusion. Of these patients, 29 were excluded for further analysis: 15 because no PSMA positive tumor lesion was detected and 14 because PET and/or CT scan was missing. Finally, imaging data of 362 men with PCa were used for analysis. The median injected activity was 98.7 MBq (range 71.2 – 184 MBq) over the entire population, with a median total administered peptide amount of 2.49 μg (range 1.06 – 5.91 μg). Furosemide (10 mg) was administered to 71.5% of the patients and iodinated contrast was used in none of the cases. The median volume of the prostatic index lesions was 9.50 mL, with an inter-quartile range of 4.11 – 20.6 mL, so the three groups we defined as: 1) ≤4.11 mL, 2) 4.11 – 20.6 mL and 3) ≥20.6 mL. Patient characteristics and demographics over the three groups are shown in Table 1.

For parotid, liver and gluteus no significant differences in tissue-to-blood ratios were observed for both SUV_{mean} and SUV_{peak} over the groups. However, the kidney-to-blood ratio of group 3 was significantly lower compared to group 1 for $SUV_{mean,}$ and SUV_{peak}, though the absolute differences between the groups were quite small. Results are shown in Fig. 1. Median SUV_{mean} and SUV_{peak} values for index lesions were 5.09 (range 1.39 – 15.9) and 7.53 (range 2.19 – 44.7) over all patients, respectively. However, 71 patients had more than one PCa lesion, and when taking into account all PCa lesions, median SUV_{mean} and SUV_{peak} were 4.78 (range 1.39 – 15.9) and 7.54 (range 2.19 – 44.7), respectively. Index lesion volume proved correlated with lesion SUV_{mean} and SUV_{peak} (both $p<0.001$, see Supplemental Fig. 1), and accordingly, tumor SUV_{mean} and SUV_{peak} significantly differed between the patient groups as is shown in Fig. 2.

Uptake versus PSMA Peptide Amount

The effect of administered peptide amount on ^{68}Ga-PSMA-11 tumor uptake per group was assessed using correlation plots and an interaction linear regression model. Results of Spearman's correlation, based on index lesions only, are presented in Fig. 3. No significant correlation between peptide amount and lesion SUV_{peak} or SUV_{mean} was observed, except for a significant positive low correlation ($p=0.008$) for SUV_{mean}

in patients with small index lesion volumes (group 1). Similar results were observed while taking into account all PCa lesions (see Supplemental Fig. 2). In addition, potential correlations of administered peptide amount on organ uptake per group were assessed (see Supplemental Fig. 3).

Linear regression model results confirmed these findings and showed that, for both SUV_{mean} and SUV_{peak}, there was no interaction effect between administered peptide amount and tumor lesion volume. In other words, the effect of administered peptide amount on SUV_{mean} and SUV_{peak} of tumor lesions was not modified by tumor lesion volume. Based on these results, receptor saturation was not deemed likely to occur in any of the groups, and thus, also not in patients with low tumor volumes as hypothesized.

For the multivariable analysis, SUV values were log transformed to correct for the non-normal distribution. Results of this multivariable analysis showed that, after exclusion of variables that appeared not significant, the variable 'BMI' had a significant positive impact on both SUV_{mean} ($p=0.0123$) and SUV_{peak} ($p=0.0300$). Variables such as age, eGFR, injection-acquisition interval, furosemide administration during scan, type of scanner, risk of PCa recurrence and disease spread did not prove relevant.

DISCUSSION

This study assessed the effect of peptide amount and index lesion volume on ^{68}Ga-PSMA-11 uptake on PET/CT in 362 primary PCa patients. Analyses showed that a larger lesion volume was indeed related to a higher tumor uptake on ^{68}Ga-PSMA-11 PET/CT, while the administered peptide amount did not shown such an evident relation. In addition, a linear regression analysis demonstrated no interaction effect between administered peptide amount and lesion volume on tumor uptake (SUV_{mean} and SUV_{peak}), meaning that index lesion volume did not modify the effect of administered peptide amount on tumor uptake. Though these results were calculated for index lesions only, the outcomes did not change when all lesions per patient were taken into account in the correlation analysis (see Supplemental Fig. 2). Differences in index lesion volume also did not result in relevant variations in normal tissue accumulation (organ to blood ratios for parotid, liver or gluteal muscle), except for kidney uptake.

The group with the smallest index lesions (volume of <4.11 mL) did reveal a significant positive low correlation between peptide amount and SUV_{mean}, however, this finding did not imply receptor saturation. In fact, if receptor saturation would have played any role, a negative correlation was hypothesized since smaller tumors are assumed to have a lower number of receptors, and hence, have less capability to bind the ^{68}Ga-PSMA-11. Therefore, it was concluded that receptor saturation did not occur in our population with primary disease (<174 mL index lesion volume) at peptide amounts between 1.06 and 5.91 µg. Substantial uptake differences might not have been expected beforehand, because of low peptide amounts with relatively small ranges in case of ^{68}Ga-PSMA-11 administration. Labelling with Fluorine-18 (^{18}F) would possibly result in even greater ranges in administered peptide amounts, since its longer half-life enables usage over longer periods of time after production. Recently, preclinical analyses have been performed regarding such ^{18}F-labeled PSMA ligands to assess potential effects of administered peptide amounts on accumulation in tumors and organs (*14, 15*). In those studies, both with ~100-fold differences in administered molar activities, lower injected molar activities resulted in reduced uptake in PSMA-expressing tissues. Unfortunately, direct translation of these results to the clinical setting remains

challenging. In PSMA-based radionuclide therapy the peptide amounts can easily be 50-times higher than for diagnostic imaging, and also in the metastatic setting tumor volumes can be far more profound than those in the current study. Extrapolation of the current results to these settings is difficult, nevertheless there are some studies that underline the relevance of peptide amounts in these settings (*16, 17*). Still, our results regarding ^{68}Ga-PSMA-11 remain important since no occurrence of receptor saturation in the diagnostic setting was confirmed.

The evaluations of ^{68}Ga-PSMA-11 accumulation in normal tissues showed that group 1 and 2 had a significant higher kidney to blood ratio compared to group 3 for SUV_{peak} and SUV_{mean}. It is hypothesized that the higher tumor uptake with increasing lesion size resulted in a lower kidney uptake and/or renal excretion. Such a tumor sink effect was indeed previously described for ^{68}Ga-PSMA-11, although these studies included PCa patients with much larger tumor lesion volumes (*18, 19*). In these studies, a tumor sink effect was also observed for organs other than kidney, such as salivary glands, spleen and liver, while this was not the case in this study. Probably, tumor volumes were too low in these primary PCa patients to achieve a decrease in normal tissue uptake. Future research is needed to also evaluate these observations for ^{177}Lu-PSMA. Although, for therapy with ^{177}Lu-PSMA I&T in metastatic castration-resistant PCa patients a simulation study by Begum *et al.* showed that increasing total tumor volumes (up to 3 L) resulted in a decrease in tumor and organ uptake (kidney, parotid glands and submandibular glands) (*17*).

In the current study, definition of PSMA-positive tumor lesion volume within the prostate was one of the important challenges. Though the use of two different strategies have been presented in the Supplemental Data, only the manual instead of semi-automatic segmentation was chosen to quantify uptake in tumor lesion. For lesions with relatively low SUV values, segmentation thresholds were set at SUV≈1, resulting in volumes that consisted of benign prostate as well as tumor tissue. Compared to manual segmentation, these automatic generated volumes overestimated actual tumor lesion volume, a finding that has been addressed before by other groups (*20*). Manual segmentation, on the other hand, is inherently to be observer dependent, but did result in a rather good Dice Similarity Coefficient of 0.73 (*21*).

All patients were divided into three groups based on their intraprostatic lesion volume to assess its relation to peptide amount. Some patient characteristics significantly differed between those groups, such as age, administered peptide amount, administered radioactivity, scanner type, risk of recurrence and disease spread (see Table 1). It was hypothesized that the higher age and classification observed with increasing lesion volume can be explained by the larger and thus more aggressive tumors (*22*). Such a similar trend of higher Gleason score and increasing age resulting in higher tumor loads was previously described by Gaertner *et al.* (*19*). For administered peptide amount, the analyses focused on a trend within ranges of this peptide amount instead of the mean differences between groups, and therefore, this significant difference between groups probably did not cause any bias. Differences in administered radioactivity were also taken into account, since SUV measures inherently correct for injected activity and patient weight. Though these significant variables were not likely to impact the conclusions, a multivariable analysis was performed to assess whether these individual parameters potentially could impact tumor uptake. BMI showed to have a positive impact on tumor uptake (SUV_{peak} and SUV_{mean}), which was expected since SUV values are corrected for weight. In addition, this result was not expected to impact our conclusions regarding the effect of peptide amount on tumor uptake compared between groups, since BMI did not significantly differ between groups.

Technical factors such as acquisition parameters (including type of scanner and acquisition time) and post-processing can also have a major impact on the quantitative indices derived from PET-images. Accordingly, harmonization of imaging protocols is highly important in studies with multiple scanner-types. This led to the limitation that partial volume effect (PVE) correction could not be applied, as this functionality is only available on the Vereos PET/CT. PVE corrections generally improve quantitative accuracy, implying that counts are recovered especially in areas of heterogeneous uptake or in small lesions (<ø2 cm). However, where PVE possibly impacted uptake differences between groups, it is unlikely that this affected the observed correlation trends within one group. This is of major importance, since one may expect the PVE to play a part especially in all lesions of group 1 (≤4.11 mL) and since tumor lesion volumes

were distributed throughout administered peptide amounts in this group, correlation trends were not affected. Still, even without PVE-correction the digital Vereos PET/CT has better performances compared to the two Gemini scanners and a better quantitative performance is known to have a larger impact on smaller lesions (i.e., group 1). This positive effect could have biased the positive correlation that was observed in this group, since a significantly larger percentage of patients in group 1 was imaged using the Vereos PET/CT compared to group 2 and 3.

Results of this study do not have direct implications on current clinical care. There is no need to adjust current on-site production procedures of ^{68}Ga-PSMA-11, since interpatient differences in administered peptide amount do not affect ^{68}Ga-PSMA-11 tumor uptake, because PSMA saturation does not occur in a population of primary PCa with small administered peptide amounts (<5.91 µg). Unfortunately, this result is not directly translatable to therapy, since it is unknown whether administrations of larger peptide amounts (~250 µg) will result in full occupation of tumor PSMA receptors. Future research is essential to investigate this peptide saturation threshold to determine potential impact on PSMA radioligand therapy. Still, this study underlined a safe administration of PSMA-11, regarding receptor saturation, at doses lower than 5.91 µg, which supports the EANM guideline with suggested maximum peptide doses of 6 µg regarding toxicity (*23*).

CONCLUSION

Overall, no evident effect was observed of administered peptide amount on ^{68}Ga-PSMA-11 uptake in prostatic tumor lesions in patients with primary PCa and this was also not dependent on lesion volume. Only for patients with small tumor lesion volumes, administered peptide amount showed a significant positive correlation with tumor SUV_{mean}. Still, these findings imply that no receptor saturation occurs in men with primary PCa after administration of peptide levels of ~2.5 µg PSMA-11**.**

Statement of Disclosure

No funds, grants or other support were received. All authors declare that they have no conflict of interest that are relevant to the content of this article.

KEY POINTS

KEY POINTS QUESTION: Can administered peptide amount affect ^{68}Ga-PSMA-11 tumor uptake and is this potential effect dependent on tumor lesion volume?

PERTINENT FINDINGS: Patients with primary prostate cancer (n = 362) that received ^{68}Ga-PSMA-11 PET/CT were selected for this retrospective study, patients were categorized in groups based on tumor lesion volume and scan data were retrospectively analyzed. Correlation and (multivariable) linear regression analyses showed that administered peptide amount did not significantly affect tumor uptake, except for a significant positive low correlation for patients with the smallest index lesions, and lesion volume did not modify the effect of administered peptide amount on tumor uptake. These findings implied that no receptor saturation occurred in this population of primary prostate cancer with small administered peptide amounts (~2.5 μg).

IMPLICATIONS FOR PATIENT CARE: Since PSMA saturation does not occur after administration of small peptide amounts, there is no need to adjust current on-site production procedures of ^{68}Ga-PSMA-11.

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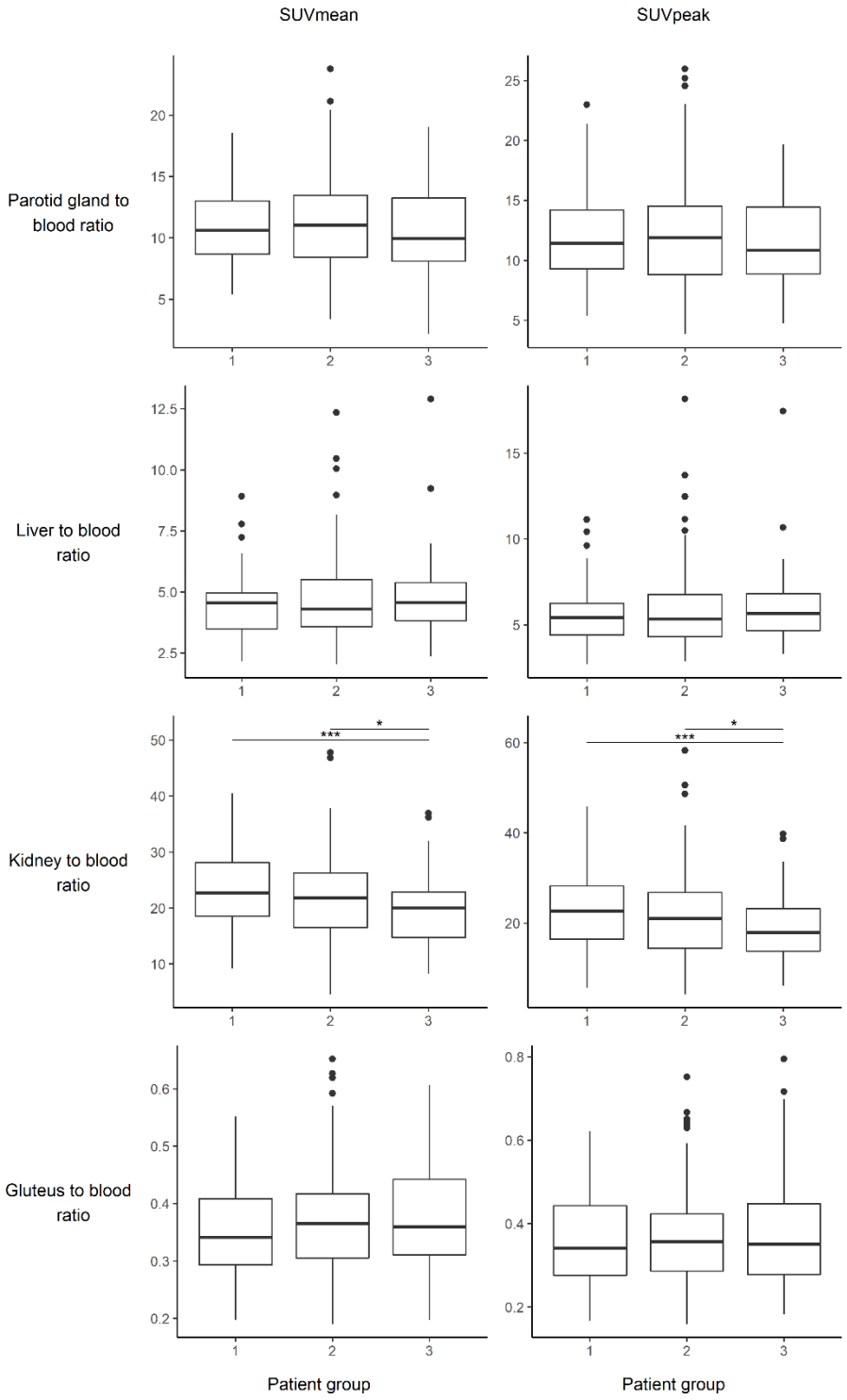

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334 **Figure 1.** Normal tissue vs blood ratios (SUV_{mean} and SUV_{peak}) for ^{68}Ga-PSMA-11 per patient group (based

335 on volume of prostatic index lesion: 1) ≤4.11 mL (≤Q1), 2) 4.11 – 20.6 mL and 3) ≥20.6 mL (≥Q3)).

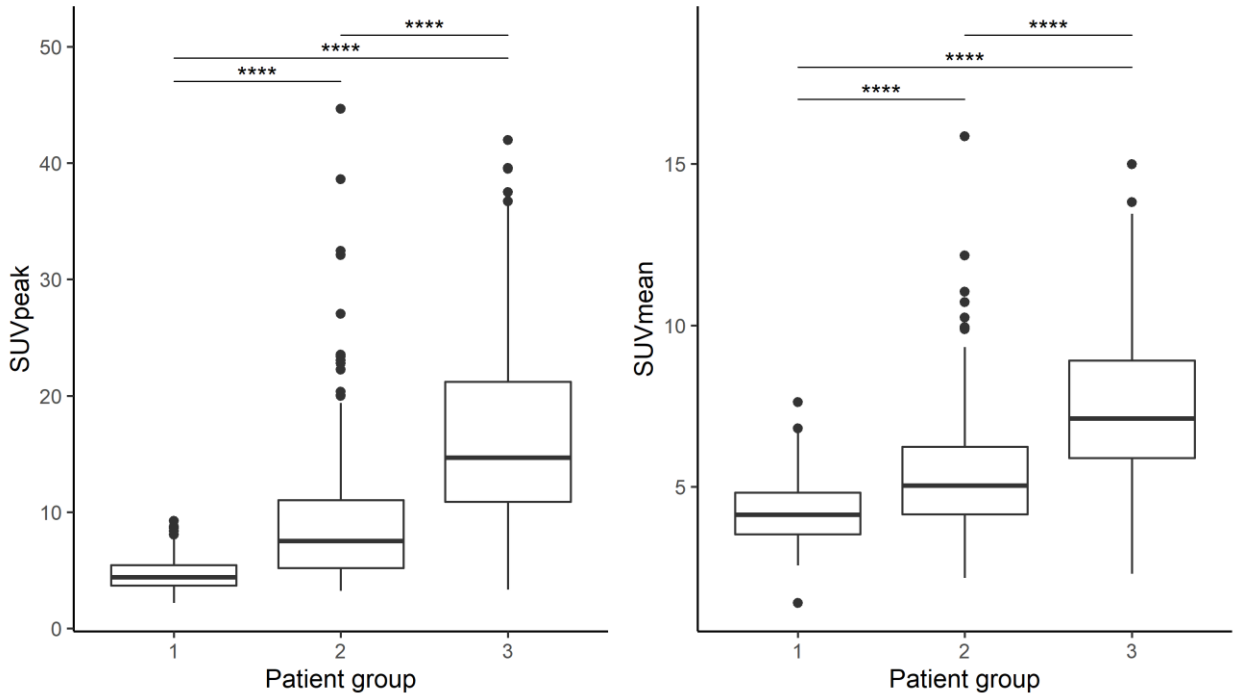
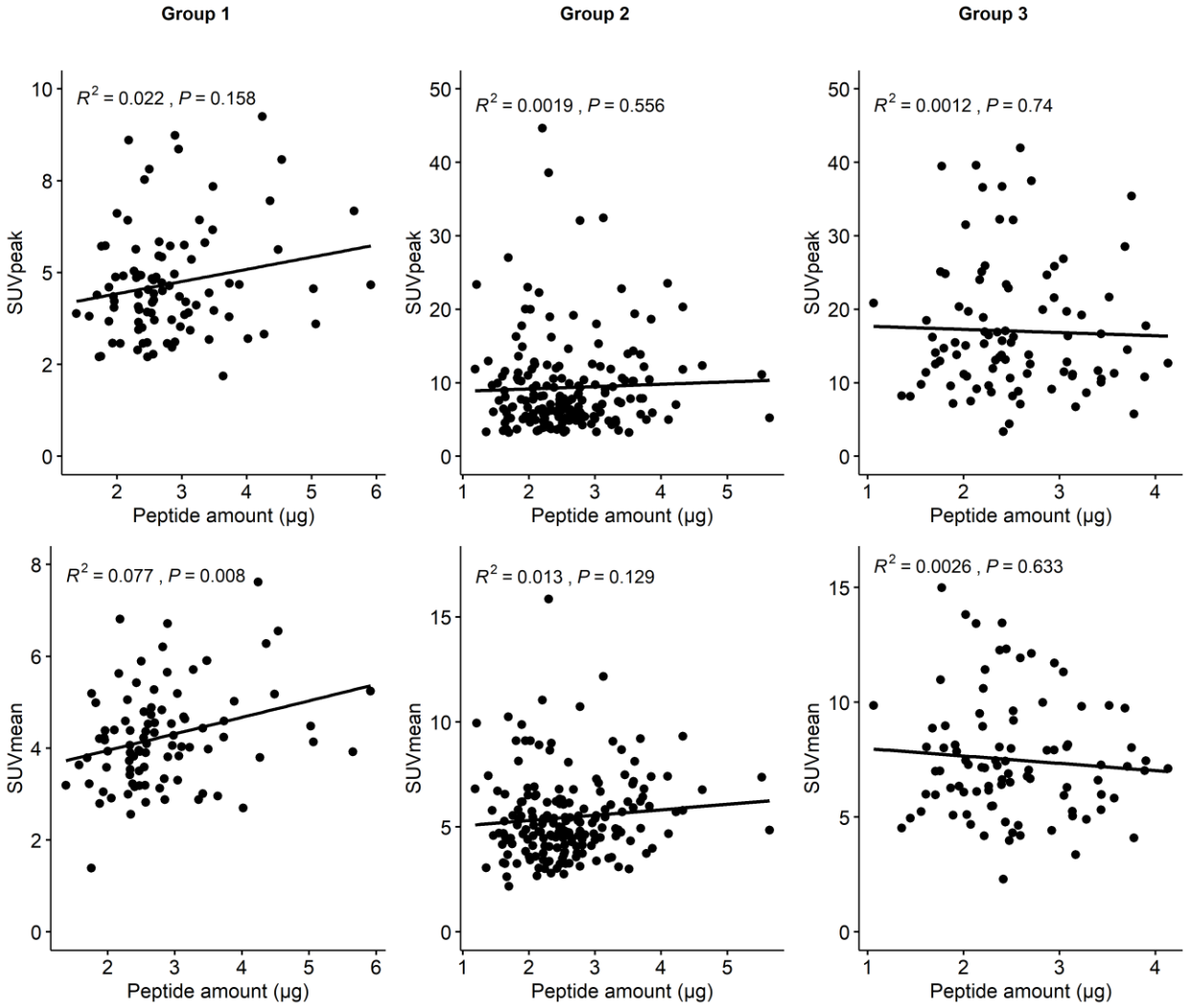


Figure 2. Boxplots of tumor ^{68}Ga-PSMA-11 uptake per group (based on volume of prostatic index lesion: 1) ≤4.11 mL (≤Q1), 2) 4.11 – 20.6 mL and 3) ≥20.6 mL (≥Q3)).



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341 **Figure 3.** Correlation plots of tumor SUV_{peak} or SUV_{mean} vs administered peptide amount (µg) per group

342 (based on volume of prostatic index lesion: 1) ≤4.11 mL (≤Q1), 2) 4.11 – 20.6 mL and 3) ≥20.6 mL (≥Q3))

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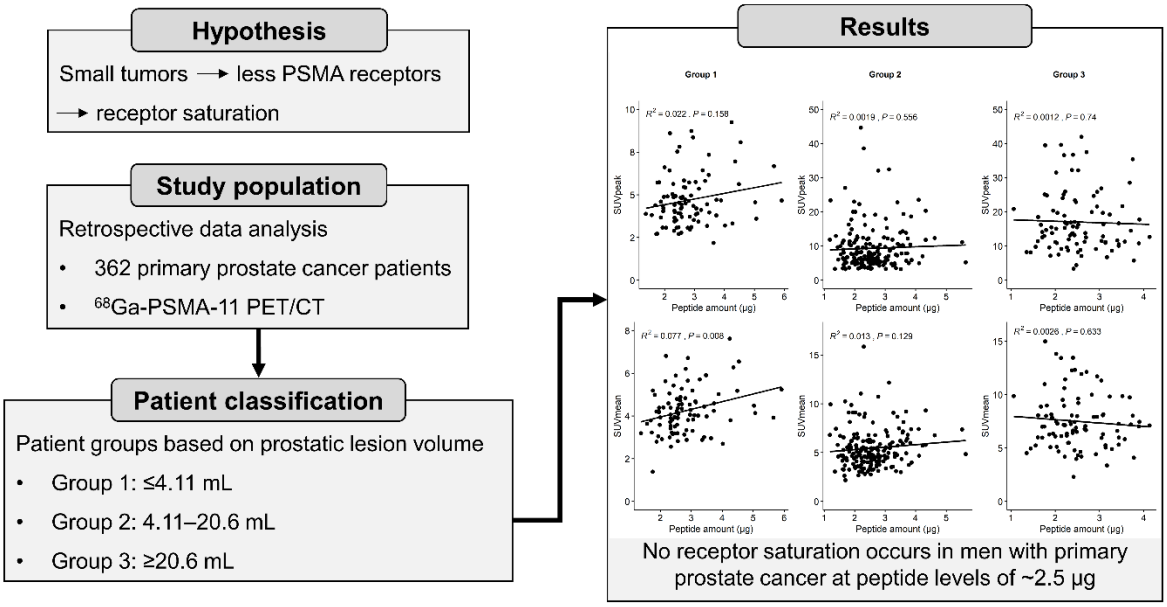
Table 1. Patient demographics and characteristics for patients receiving ^{68}Ga-PSMA-11 per group based on lesion volume: 1) ≤4.11 mL (≤Q1), 2) 4.11 – 20.6 mL and 3) ≥20.6 mL (≥Q3))

	Group 1	**Group 2**	**Group 3**	**p value**
N	91	180	91	
Age (years)	67±6	69±7	68±8	0.044
BMI (kg/m^2)	26.6±3.50	26.0±3.30	26.8±3.81	0.151
eGFR (mL/min/1.73 m^2)	76.6±13.1	76.5±16.1	76.5±20.8	0.999
Injection-acquisition interval (min)	58±11	59±11	60±9	0.379
Administered peptide amount (µg)	2.83±0.88	2.58±0.76	2.52±0.66	0.015
Injected radioactivity (MBq)	111.0±24.9	101.1±17.9	98.6±11.0	<0.001
Furosemide during scan	51 (56%)	138 (77%)	70 (77%)	0.001
Scanner type - Gemini TF Big Bore - Gemini TF - Vereos PET/CT	 37 (41%) 21 (23%) 33 (36%)	 92 (51%) 56 (31%) 32 (18%)	 55 (60%) 25 (28%) 11 (12%)	<0.001
Risk of recurrence - Intermediate - High	 35 (39%) 56 (61%)	 22 (12%) 158 (87%)	 3 (3%) 88 (97%)	<0.001
Disease spread - Local - Locally advanced - Oligometastatic - Metastatic	 64 (70%) 26 (29%) 1 (1%) 0 (0%)	 71 (39%) 82 (46%) 24 (13%) 3 (2%)	 9 (10%) 45 (50%) 28 (31%) 9 (10%)	<0.001

Abbreviations: eGFR: estimated glomerular filtration rate; BMI: body mass index. Continuous variables are shown as mean ± standard deviation and categorical variables as number (%).

350 **Graphical abstract**



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Supplemental Data

Tumor segmentation

Methods

Since there still is no gold standard for prostate tumor segmentation on PSMA-PET, segmentation was performed using two methods; a semi-automatic 20% SUV_{max} threshold based segmentation and manual contouring at fixed window level of $SUV_{min-max}$ 0-5 (*1*). Both methods were conducted independently by two observers and an interim analysis was completed after segmentation of the first 50 patients was completed by both observers. Dice Similarity Coefficients (DSC) were calculated (*2*) and the most suitable and accurate approach for lesion segmentation was selected for the remaining patients.

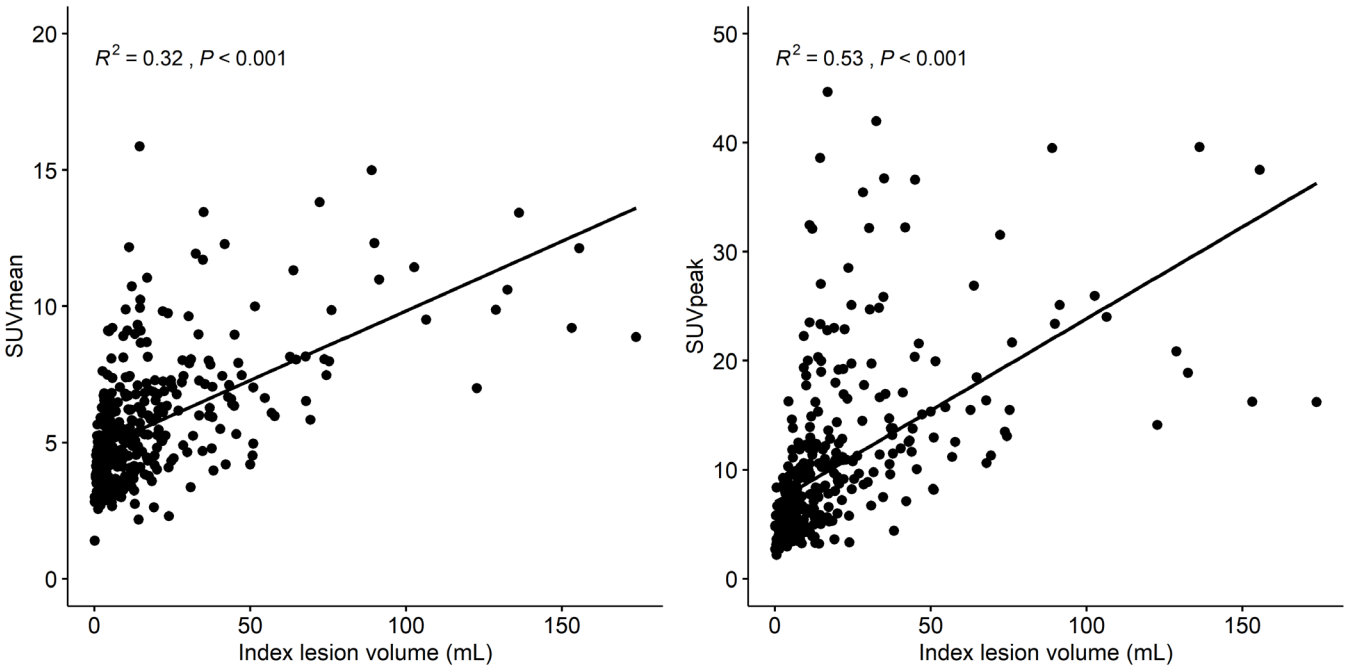
Results

Interim analysis of 50 patients resulted in dice coefficients of 0.81±0.12 and 0.73±0.12 for the threshold based and manual segmentation, respectively. Due to its semi-automatic nature, the threshold based segmentation method naturally resulted in higher DSC values. However, it often produced unrealistic tumor volumes compared to manual segmentation and therefore the latter method was used for further analysis.

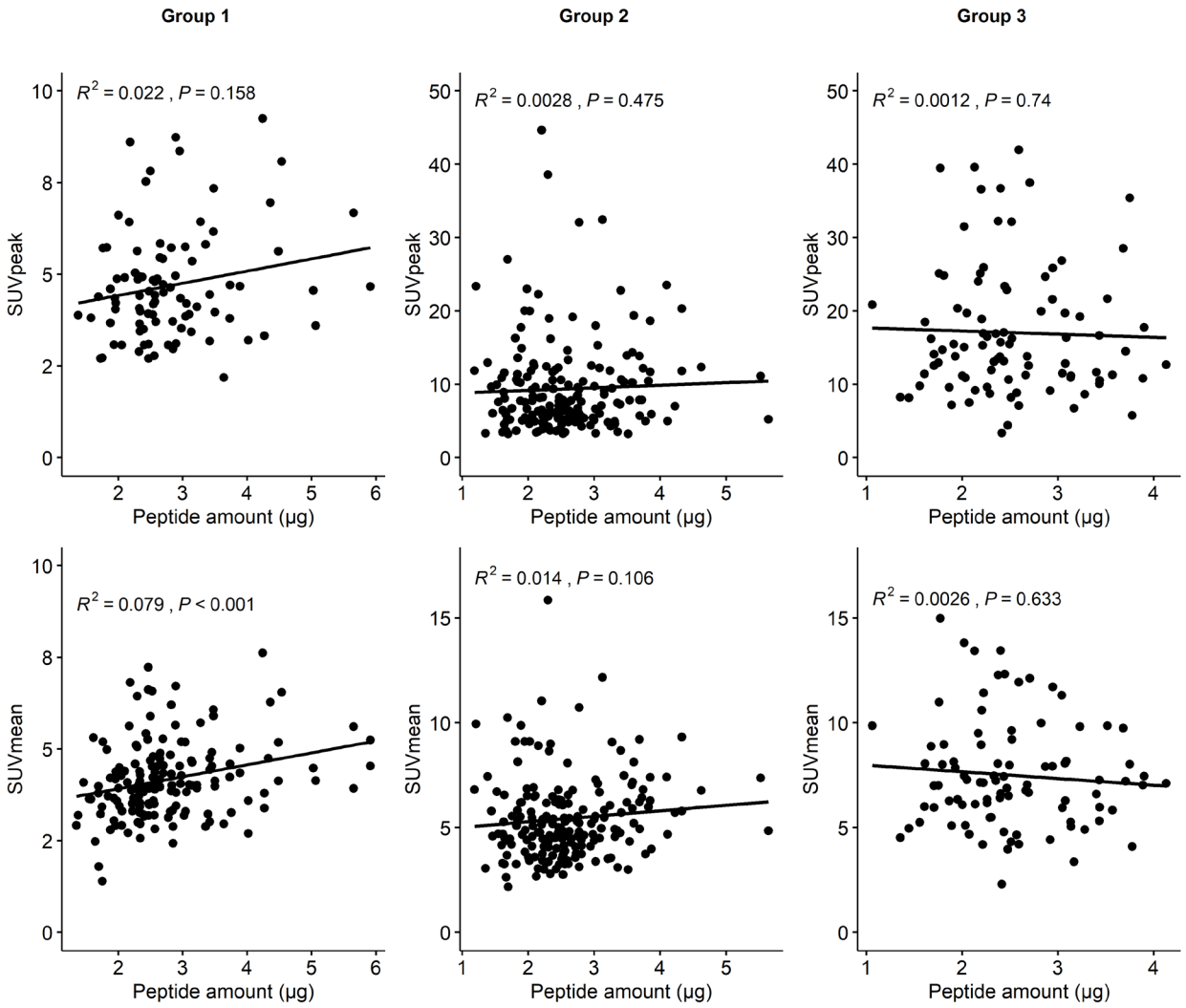
References

1. Zamboglou C, Fassbender TF, Steffan L, et al. Validation of different PSMA-PET/CT-based contouring techniques for intraprostatic tumor definition using histopathology as standard of reference. *Radiother Oncol*. 2019;141:208-13.

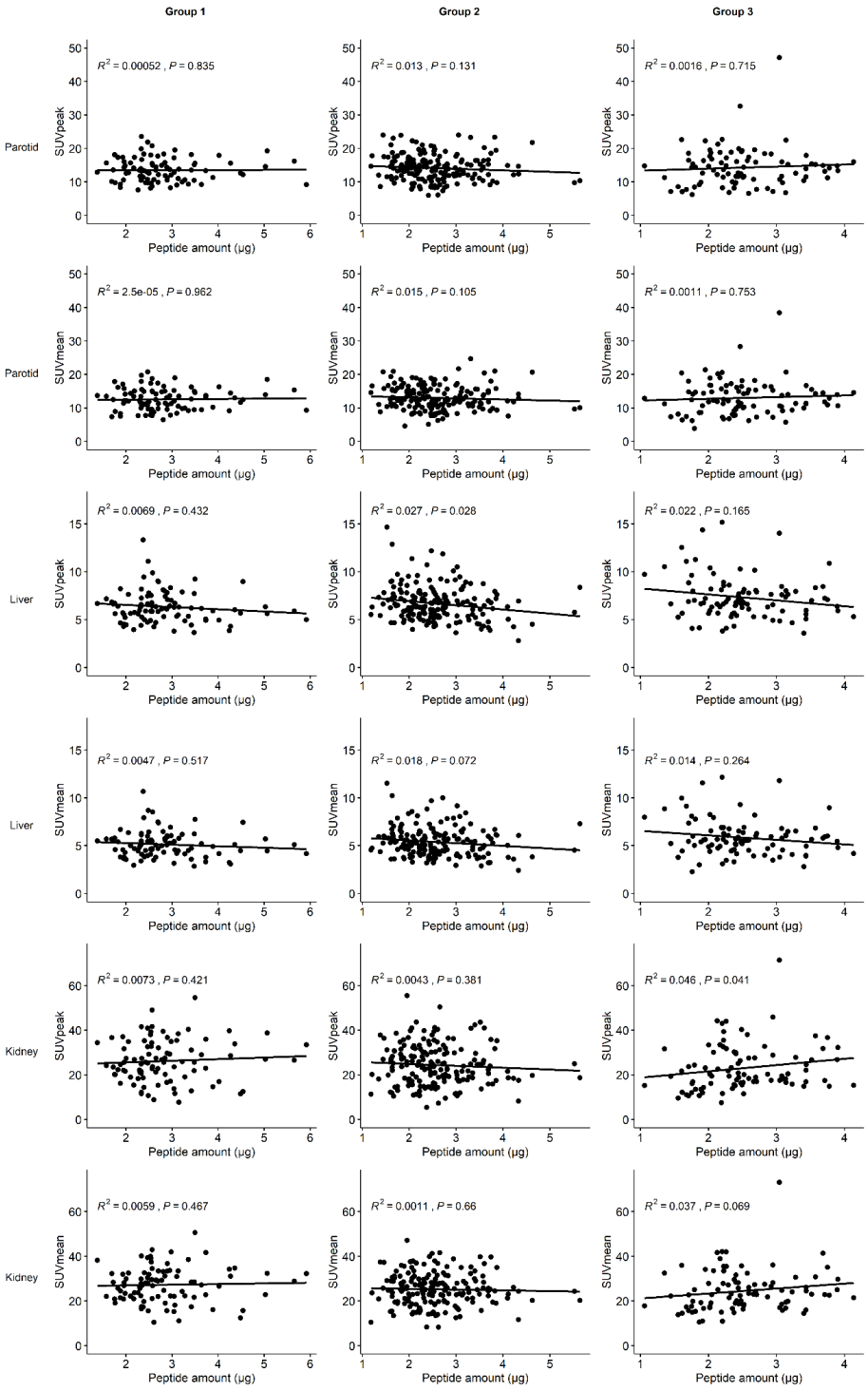
2. Dice LR. Measures of the amount of ecologic association between species. *Ecology*. 1945;26:297-302.



Supplemental Figure 1 – Spearman's correlations plots showed that index lesion volume was significantly correlated with tumor uptake (for SUV_{mean} and SUV_{peak}) of ^{68}Ga-PSMA-11 in primary prostate cancer patients.



Supplemental Figure 2 – Correlation plots of tumor SUV_{peak} or SUV_{mean} vs administered peptide amount (µg) per group taking into account all prostate cancer lesions (based on volume of prostatic index lesion: 1) ≤4.11 mL (≤Q1), 2) 4.11 – 20.6 mL and 3) ≥20.6 mL (≥Q3))



Supplemental Figure 3 – Correlation plots of organ SUV_{peak} or SUV_{mean} vs administered peptide amount (µg) per group (based on volume of prostatic index lesion: 1) ≤4.11 mL (≤Q1), 2) 4.11 – 20.6 mL and 3) ≥20.6 mL (≥Q3))**.**