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PSA-stratified performance of ^{18}F- and ^{68}Ga-labeled tracers in PSMA-PET imaging of patients with biochemical recurrence of prostate cancer

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ABSTRACT

Purpose: Several studies outlined the sensitivity of ^{68}Ga-labeled PET tracers against the prostate-specific membrane antigen (PSMA) for localization of relapsed prostate cancer in patients with renewed increase in the prostate-specific antigen (PSA), commonly referred to as biochemical recurrence. Labeling of PSMA tracers with ^{18}F offers numerous advantages, including improved image resolution, longer half-life and increased production yields. The aim of this study was to assess the PSA-stratified performance of the ^{18}F-labeled PSMA tracer ^{18}F-DCFPyL and the ^{68}Ga-labeled reference ^{68}Ga-PSMA-HBED-CC. **Methods:** We examined 191 consecutive patients with biochemical recurrence according to standard acquisition protocols with ^{18}F-DCFPyL (*N*=62, 269.8 MBq, PET scan at 120 minutes p.i.) or ^{68}Ga-PSMA-HBED-CC (*N*=129, 158.9 MBq, 60 minutes p.i.). We determined PSA-stratified sensitivity rates for both tracers and corrected our calculations for Gleason scores using iterative matched-pair analyses. As an orthogonal validation, we directly compared tracer distribution patterns in a separate cohort of 25 patients, sequentially examined with both tracers. **Results:** After prostatectomy (*N*=106), the sensitivity of both tracers was significantly associated with absolute PSA levels ($P=4.3\times10^{-3}$). Sensitivity increased abruptly, when PSA values exceeded 0.5µg/L ($P=2.4\times10^{-5}$). For PSA <3.5µg/L, most relapses were diagnosed at a still limited stage ($P=3.4\times10^{-6}$). For PSA of 0.5-3.5µg/L, PSA-stratified sensitivity was 88% (15/17) for ^{18}F-DCFPyL and 66% (23/35) for ^{68}Ga-PSMA-HBED-CC. This significant difference was preserved in the Gleason-matched-pair analysis. Outside of this range, sensitivity was comparably low (PSA <0.5µg/L) or high (PSA >3.5µg/L). After radiotherapy (*N*=85), tracer sensitivity was largely PSA-independent. In the 25 patients examined with both tracers, distribution patterns of ^{18}F-DCFPyL and ^{68}Ga-

PSMA-HBED-CC were strongly comparable (P=2.71x10^{-8}). However, in 36% of the PSMA-positive patients we detected additional lesions on the ^{18}F-DCFPyL scan (P=3.7x10^{-2}). **Conclusion:** Our data suggest that ^{18}F-DCFPyL is non-inferior to ^{68}Ga-PSMA-HBED-CC, while offering the advantages of ^{18}F-labeling. Our results indicate that imaging with ^{18}F-DCFPyL may even exhibit improved sensitivity in localizing relapsed tumors after prostatectomy for moderately increased PSA levels. Although the standard acquisition protocols, used for ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC in this study, stipulate different activity doses and tracer uptake times after injection, our findings provide a promising rationale for validation of ^{18}F-DCFPyL in future prospective trials.

Keywords

PSMA ligands, prostate cancer, biochemical recurrence, ^{68}Ga-PSMA-HBED-CC, ^{18}F-DCFPyL

INTRODUCTION

After radical prostatectomy, concentrations of prostate-specific antigen (PSA) typically decrease below detection threshold. A renewed increase in PSA levels to above 0.2µg/L - commonly referred to as biochemical recurrence (BCR) - signifies a potential relapse of prostate cancer after surgery (*1*). Similarly, BCR is defined as a PSA increase of at least 2µg/L above the minimum PSA level after radiotherapy. The prostate-specific membrane antigen (PSMA) is particularly overexpressed on the surface of prostate cancer cells (*2*). Tracers for positron emission tomography (PET), that bind specifically to PSMA, have gained increasing attention for localization of tumors in BCR patients (*3-12*). In particular, ^{68}Ga-PSMA-HBED-CC displays substantial sensitivity in detecting tumor relapse after prostatectomy, even when PSA levels are low (≥0.2µg/L) (*3-9*). Furthermore, four studies have consistently shown that ~95% of the ^{68}Ga-PSMA-HBED-CC-positive lesions have histological tumor correlates in biopsies and surgical resections (*13-16*).

The short half-life of ^{68}Ga makes ^{68}Ga-PSMA-HBED-CC inconvenient for longer transport, so that cost-intensive, local Gallium generators are required, which typically have lower yields at the end of their first half-life (*17*). Furthermore, the resolution of ^{68}Ga-labeled tracers is physically limited due to positron range effects (*18*). In contrast, ^{18}F labels avoid these intrinsic difficulties and can be produced at high yields in central cyclotrons. In 2011, first preclinical data with the ^{18}F-labeled PSMA ligand DCFPyL were published (*10*). Recently, two proof-of-principle studies demonstrated the general capability of ^{18}F-DCFPyL to detect relapsed tumors in 9 (*11*) and 14 BCR patients (*12*), respectively. Here, we examined 62 BCR patients with ^{18}F-DCFPyL and benchmarked

the PSA-stratified sensitivity of ^{18}F-DCFPyL against ^{68}Ga-PSMA-HBED-CC (*N*=129 patients).

MATERIALS AND METHODS

Patients

We examined 191 consecutive prostate cancer patients with BCR after radical prostatectomy (*N*=106) or radiotherapy (*N*=85), either with ^{18}F-DCFPyL (*N*=62) or ^{68}Ga-PSMA-HBED-CC (*N*=129) (Table 1). Patients were selected according to the following criteria.

Prostatectomy cohort:

- Complete removal of the entire prostate gland, R0- or R1- resection
- Recent PSA increase to ≥0.2µg/L after nadir

Radiotherapy cohort:

- Organ-preserving local treatment (external beam radiation therapy, brachytherapy, seed implantation, high-intensity focused ultrasound)
- PSA increase of at least 2.0µg/L above the minimum PSA value after therapy, as determined by the referring urologist

Additionally, we required that no distant metastases had been detected in previous examinations, patients did not receive anti-androgen therapy and a time period of at least 6 months had elapsed between the initial therapy and the PET scan. We note that 47 of our prostatectomy patients received additional salvage radiotherapy before the PET scan (16 ^{18}F-DCFPyL cases, 31 ^{68}Ga-PSMA-HBED-CC cases).

As an additional approach, we continued our efforts of a previous study (*12*) (*N*=14 patients) and sequentially examined prostate cancer patients with ^{68}Ga-PSMA-HBED-

CC and ^{18}F-DCFPyL. This allowed a direct comparison of the tracer distribution pattern within a separate validation cohort of 25 patients. Inclusion criteria for this validation cohort were described previously (*12*).

This study was conducted in accordance with the Institutional Review Board. All patients gave written informed consent to PET imaging and inclusion of their data in a retrospective analysis. All procedures were performed in compliance with the regulations of the responsible local authorities (District Administration of Cologne, Germany).

Imaging

Synthesis of PSMA-PET tracers was performed as previously described for ^{18}F-DCFPyL (*10,12*) and ^{68}Ga-PSMA-HBED-CC (*19,20*). Each week, we produced three independent batches of ^{68}Ga-PSMA-HBED-CC and one batch of ^{18}F-DCFPyL. Patients were randomly assigned by tracer availability. Images were acquired on a Biograph mCT Flow^{TM} (Siemens) PET/CT scanner. In accordance with standard acquisition protocols (*3,5,7,12*), patients fasted for at least 4 hours, prior to intravenous injection of ^{68}Ga-PSMA-HBED-CC (158.9±45.1 MBq) or ^{18}F-DCFPyL (269.8±81.8 MBq). PET images were acquired one or two hours after injection, respectively. The same filters and acquisition times were used for both tracers. Non-contrast-enhanced (low-dose) CT scans were acquired in parallel to PET imaging. Images were reconstructed based on the ultra-high definition algorithm (*21*).

PSMA-PET scans were analyzed through visual inspection by at least one specialist in nuclear medicine and one specialist in radiology. A scan was scored as *positive*, if focal tracer accumulation was detected in the prostate fossa, in a lymph node or at a distant site. To be interpreted as a PET-positive lymph node, we required a

morphological correlate on the CT scan. A tumor relapse was interpreted as *limited*, if tracer accumulation was limited to the prostate fossa or to locoregional lymph nodes. Otherwise, a positive scan was scored as *advanced.* Furthermore, we scored PET-positivity based on the number of PET-positive lesions (score 0: negative scan, score 1: one lesion, score 2: two lesions, score 3: more than two positive lesions).

Confirmation of PET-Positive Lesions through Biopsies

As an external validation of our imaging results, 15 BCR patients with PET-positive tissue within the prostate fossa were subjected to biopsy (4 ^{18}F-DCFPyL and 11 ^{68}Ga-PSMA-HBED-CC cases). For each patient, we obtained 12 local biopsies from the residual prostate gland (13 radiotherapy patients) or prostate fossa (2 prostatectomy patients), guided by ultrasound. We compared histology with tracer distribution and categorized the findings as follows:

- full concordance - all PSMA-positive segments histologically confirmed, all PSMA-negative segments tumor-free
- partial concordance - all PSMA-positive segments histologically confirmed, but not all PSMA-negative segments tumor-free
- false positive cases - at least one PSMA-positive segment lacking histological confirmation
- full discordance - PSMA-positive segments lacking histological confirmation, PSMA-negative segments infiltrated by tumor cells.

Statistics and Mathematical Modeling

For PSA stratification, we systematically calculated tracer sensitivity within multiple small PSA ranges. Each PSA interval was characterized in terms of its center and width. Based on the detection rate within each interval, we compiled a PSA-stratified tracer sensitivity curve without any a-priori assumption of PSA thresholds. Analogously, we derived curves displaying the PSA-stratified rate of diagnosis at limited stage based on multiple small PSA intervals. From these curves, we derived a diagnostic window as follows: The lower threshold was determined as the PSA level, where sensitivity exceeded 50%. The upper threshold was taken as the PSA level, where the fraction of limited relapses detected decreased 50%.

In order to correct our sensitivity comparison between ^{18}F- and ^{68}Ga-labeled tracers for Gleason scores, we randomly selected matched sub-cohorts of 30 ^{18}F and 30 ^{68}Ga tracer patients with pairwise equal Gleason scores (1,000 iterations). We then determined the log-transformed ratio between PSA-stratified sensitivity in the ^{18}F sub-cohort and sensitivity in the ^{68}Ga sub-cohort for each iteration. We thus obtained log-transformed ratios r_i, with $r_i > 0$, if ^{18}F sensitivity was superior. Finally, we compared r_i against the null hypothesis by a paired t-test.

RESULTS

PSA Levels Predict Sensitivity of PSMA Imaging after Prostatectomy

We first subdivided the 191 consecutive PET scans into the following groups:

- PSMA-negative scans (*N*=43)
- PSMA-positive scans displaying *limited* relapse (*N*=85)
- PSMA-positive scans displaying *advanced* relapse (*N*=63)

In prostatectomy patients, PSA levels significantly differed between these three groups (Supplemental Fig. 1A): PSA levels were significantly lower in PET-negative patients compared with PET-positive patients displaying limited (P=4.3x10^{-3}) or advanced relapse (P=4.9x10^{-7}). Furthermore, PSA levels differed significantly between limited and advanced staged relapses (P=7.6x10^{-3}). Interestingly, PSA values did not differ significantly after radiotherapy (Supplemental Fig. 1B). Intriguingly, PSMA accumulated exclusively in locoregional nodes for 75% of prostatectomy patients with limited relapse, which was rarely observed after radiotherapy (17%, P<1x10^{-4}). Most radiotherapy patients with limited relapse displayed tracer accumulation exclusively in the local tumor bed (68%), which was only occasionally detected after prostatectomy (21%, P<1x10^{-4}).

We next plotted PSA levels against the rate of PET-positive patients after prostatectomy. Furthermore, we plotted PSA against the fraction of scans, displaying a relapse at limited stage. Detection rates increased abruptly and significantly (P=2.4x10^{-5}), when PSA concentrations exceeded 0.5µg/L and remained largely unchanged above this threshold (Fig. 1A). PSA levels tightly anti-correlated with the probability of detecting cancer relapse at limited stage (Fig. 1B): While most relapsed tumors were diagnosed at limited stage for PSA <3.5µg/L, this pattern significantly (P=3.4x10^{-6}) reversed, when PSA levels exceeded 3.5µg/L. Interestingly, these rates were largely PSA-independent for PSA levels up to 25µg/L in the radiotherapy cohort (Figs. 1C and 1D). While sensitivity was high across all PSA concentrations examined (median: 91.5%), the fraction of limited stage diagnoses varied stably around a median of 67.5% for PSA up to 25µg/L.

As an external validation of our imaging results, biopsy and corresponding PET scan were concordant in 86.6% (13/15) of our patients (fully concordant: 73.3%, partially concordant: 13.3%, Supplemental Fig. 2).

^{18}F-DCFPyL Displays Improved Sensitivity in Localization of Relapsed Tumors after Prostatectomy

We plotted PSA-stratified sensitivity curves separately for ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC, and noted that the sensitivity curve of ^{18}F-DCFPyL was discretely, but robustly, shifted towards lower PSA concentrations. For PSA levels around 0.45µg/L, local sensitivity of ^{18}F-DCFPyL reached 62%, whereas ^{68}Ga-PSMA-HBED-CC detected tumor relapses in 33% of the cases (Supplemental Fig. 3, arrows). The PSA-stratified sensitivity curve of ^{18}F-DCFPyL exceeded the ^{68}Ga-PSMA-HBED-CC curve significantly (P=3.4x10^{-3}) and substantially (average sensitivity: 80% vs. 68%) for PSA of 0.5-3.5µg/L.

As an orthogonal approach, we counted the absolute number of PET-positive patients, as detected with either ^{18}F-DCFPyL or ^{68}Ga-PSMA-HBED-CC (Supplemental Table 1). For PSA of 0.5-3.5µg/L, we observed significantly (P=0.042, one-tailed Chi-square test without Yates' correction) and substantially (88.2% vs. 65.7%) more relapsed patients with ^{18}F-DCFPyL (15/17) than with ^{68}Ga-PSMA-HBED-CC (23/35). Outside of this PSA range, ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC displayed similar sensitivity (Fig. 2).

We next aimed to formally exclude the possibility that the substantial sensitivity differences between the two tracers might derive from differences in Gleason scores. We performed a mathematical confounder correction and randomly selected 30

Gleason-matched pairs from the ^{18}F and ^{68}Ga tracer cohorts of prostatectomy patients (1,000 iterations) (Supplemental Fig. 4). We then compared PSA-stratified sensitivity of the ^{18}F- and ^{68}Ga-labeled tracers for each iteration. Superiority of ^{18}F-DCFPyL was preserved in 92.1% (all PSA levels, P=5.3x10^{-234}, paired t-test against null hypothesis) and 100% (PSA <1µg/L, P=2.5x10^{-294}) of the iterations, respectively (Supplemental Figs. 5A and 5B). As a negative control, we randomly swapped tracer labels between matched pairs and found that random relabeling entirely abrogated the significant sensitivity difference between the two tracers (Supplemental Figs. 5C and 5D). This suggests that slight differences in Gleason scores cannot sufficiently account for the sensitivity differences between ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC.

A Direct Comparison of Tracer Distribution Patterns in a Validation Cohort

We continued our efforts of a recent pilot study (*12*) and sequentially examined patients with ^{68}Ga-PSMA-HBED-CC and ^{18}F-DCFPyL. This gave us a cohort of 25 patients, who had been scanned with both tracers (Fig. 3A). Pairwise comparison of matched PET scans revealed that tracer distribution patterns were highly concordant (P=2.71x10^{-8}), substantiating the validity of ^{18}F-DCFPyL. In particular, all 23 PET-positive lesions on ^{68}Ga scans could be confirmed on the corresponding ^{18}F scan (Fig. 3B).

Intriguingly, for 36% of the ^{68}Ga-PSMA-HBED-CC-positive scans (4/11) we detected additional PET-positive lesions on the ^{18}F-DCFPyL scan (Figs. 3C and 3D). In particular, PET positivity scores were significantly higher for the ^{18}F-DCFPyL scans compared with their corresponding ^{68}Ga-PSMA-HBED-CC scans (P=4.2x10^{-2}) (Figs. 3B and C). Significance increased when only the matched PET scans of the 11 ^{68}Ga-positive patients were included in the analysis (P=3.7x10^{-2}) (Figs. 3B and D).

We next asked whether we could identify any obvious reasons for the increased sensitivity of ^{18}F-DCFPyL. As exemplarily shown in Figure 4, we observed a substantial extinction of tracer signal between the kidneys in ^{68}Ga-PSMA-HBED-CC scans, most likely due to the higher activity of ^{68}Ga-PSMA-HBED-CC in the kidneys, compared with ^{18}F-DCFPyL (*12*). This might explain, why this artifact was only marginally present in the corresponding ^{18}F-DCFPyL scan (patient #1). PET-positive lesions could therefore not be reliably excluded between the kidneys on some ^{68}Ga-PSMA-HBED-CC scans. For instance, patient #2 revealed an ^{18}F-DCFPyL-positive metastasis in LVB2, which was largely annihilated in the ^{68}Ga-PSMA-HBED-CC scan (Fig. 4). Additionally, for some patients the visibility of PET-positive lesions was substantially lower with ^{68}Ga-PSMA-HBED-CC, compared with ^{18}F-DCFPyL (patient #3).

DISCUSSION

PSA Levels Pinpoint Optimized Timing of PSMA-PET

Accurate timing of PSMA-PET substantially affects its diagnostic value in BCR patients. When PET scans are acquired too early, tumor detection rates are typically low. When PSMA-PET scans are acquired too late, the number of patients identified at limited-stage disease is low, thus limiting its value for the individual patient. Our retrospective analyses suggest that the diagnostic value of PET depends on the absolute PSA level of BCR after prostatectomy, in marked contrast to the situation after radiotherapy. We established that a narrow PSA range of 0.5-3.5µg/L allows optimal detection of cancer relapse after surgery. Thus, narrow monitoring of PSA values could well be useful for accurate timing of PET imaging.

A recent study reported a sensitivity rate of 85% for ^{68}Ga-PSMA-HBED-CC in prostatectomy patients with BCR (*7*), which is fully concordant with our own observations. However, as that study did not subdivide the patient group with PSA <1µg/L, lower detection thresholds cannot be derived. Another study reported a detection rate of 58% for patients with PSA <0.5µg/L (*5*). A third retrospective study on ^{68}Ga-PSMA-HBED-CC stratified patients using fixed PSA thresholds (*6*); sensitivity was 65% (0.2-0.29µg/L), 44% (0.3-0.49µg/L) and 71% (0.5-0.99µg/L). This wide variability of tracer sensitivity suggests that results may be dependent on the patient cohorts examined, on the PSA thresholds selected and on reader-dependent differences. We therefore compared ^{18}F- and ^{68}Ga-labeled PSMA tracer cohorts that had been simultaneously examined by the same readers at the same institution and within the same time period. Furthermore, in contrast to previous studies, we employed a mathematical model, which determined tracer sensitivity for variable PSA thresholds. This enabled us to derive a PSA range, optimized for PSMA-PET imaging, without a-priori definition of PSA thresholds.

Sensitivity of PSMA-PET Parallels PSMA Expression

Mannweiler and colleagues profiled PSMA expression in 51 metastasized prostate cancer patients (*2*). They found that 96% of primary tumors and 84% of the corresponding metastases displayed detectable PSMA levels. We therefore speculated that PSMA-PET sensitivity might generally be limited to ~84%, due to heterogeneous PSMA expression in prostate cancer (*2*). Concordantly, PET imaging reached a sensitivity rate of 89% in our prostatectomy patients with PSA >3.5µg/L. Furthermore, we found surprisingly high PSA levels >2.5µg/L in 21% of the PSMA-negative patients, which can most likely be attributed to PSMA-negative metastases. We note that ^{18}F-

DCFPyL reached a sensitivity of 88% in prostatectomy patients even for PSA levels of 0.5-3.5µg/L. We thus speculate that ^{18}F-DCFPyL exploits the full potential of PSMA tracers for PSA ≥0.5µg/L.

^{18}F-DCFPyL Allows Early Localization of Cancer Relapse

Based on 62 ^{18}F-DCFPyL scans, this study confirmed previous pilot studies (*11,12*), which reported that ^{18}F-DCFPyL sensitivity is at least non-inferior to the ^{68}Ga-PSMA-HBED-CC standard. Intriguingly, in this larger study, ^{18}F-DCFPyL displayed even improved detection rates in prostatectomy patients with PSA levels of 0.5-3.5µg/L. We used matched pair analyses to exclude the possibility that Gleason scores might impact on differences in tracer sensitivity. Furthermore, we performed cross-sectional imaging of 25 patients with both tracers and found that ^{18}F-DCFPyL detected significantly more PET-positive lesions. As this improved sensitivity of ^{18}F-DCFPyL was thus consistent across three independent approaches, our results suggest that it would be well worth validating ^{18}F-labeled PSMA tracers in future prospective trials.

Limitations

We derived the lower PSA threshold (0.5µg/L) retrospectively from our PET imaging results. Below this threshold, our tracer sensitivity rates were substantially lower than the results of previous studies on ^{68}Ga-PSMA-HBED-CC for PSA levels of 0.2-0.5µg/L (*5,6*). One explanation for this incongruence might lie in our scoring system, whereby a PET-positive lymph node required a morphological correlate in the corresponding low-dose CT scan. Thus, we cannot formally exclude the possibility that PET-positive lymph nodes without a clear CT-morphological correlate were

misinterpreted as physiological tracer accumulation in the ureter or intestine. Hence, inter-observer variability may impact on the lower PSA threshold. Similarly, we defined the upper PSA threshold (3.5µg/L) based on our PET imaging results. Although none of our PET-negative patients displayed suspect lesions on the corresponding CT scan, we cannot rule out the possibility that some patients diagnosed as limited stage carried additional PET-negative lesions at distant sites.

The standard tracer acquisition protocols (*3,5,7,12*), used in this study, included different tracer uptake time periods for ^{18}F-DCFPyL (120 minutes) and ^{68}Ga-PSMA-HBED-CC (60 minutes) prior to image acquisition, due to the shorter half-life of the ^{68}Ga label. This time difference of 60 minutes may contribute to the increased sensitivity of ^{18}F-DCFPyL, as a recent pilot study observed that delayed image acquisition improves the quality of ^{68}Ga-PSMA-HBED-CC scans for four patients (*8*). Furthermore, standard acquisition protocols (*3,5,7,12*) recommend different activity dosages for ^{18}F-DCFPyL (250 MBq) and ^{68}Ga-PSMA-HBED-CC (150 MBq), due to the small yields of local Gallium generators in routine diagnostic procedures. Perhaps not surprisingly, a recent pilot study (*12*) thus reported significantly higher SUV_{max} values in tumor lesions for ^{18}F-DCFPyL compared with ^{68}Ga-PSMA-HBED-CC, which enhances the tumor-to-background ratio of ^{18}F-DCFPyL scans and might thus facilitate their interpretation. Although challenging in routine diagnostics, a potential improvement of the acquisition protocol for ^{68}Ga-PSMA-HBED-CC might thus be prolongation of the acquisition time for PET scans. We note that our uptake time periods and tracer dosages are in full concordance with standard acquisition protocols of previous studies (*3,5,7,12*). Furthermore, a large prospective trial performed PET scans with ^{68}Ga-PSMA-HBED-CC after an even shorter time period of 45 minutes, but still observed high sensitivity (*6*).

Hence, optimized acquisition protocols for PSMA tracers remain a current matter of debate.

An additional factor, contributing to the increased sensitivity of ^{18}F-DCFPyL, was the reduced tracer signal extinction between the kidneys, which we observed for ^{18}F-DCFPyL. A recent study reported that the quality of ^{68}Ga-PSMA-HBED-CC scans could be improved by delayed imaging after forced diuresis (*9*). In this study we did not employ forced diuresis, although this might well provide a means of improving the performance of both tracers.

Histological validation was available only for a relatively small sub-cohort of patients. Hence, tracers could not be compared on the basis of histopathological results and we cannot formally exclude the possibility that additional ^{18}F-DCFPyL-positive lesions represent false-positive results. Furthermore, we obtained local biopsies exclusively from the prostate fossa of PET-positive patients with limited relapse. Consequently, PET-negative and advanced-staged patients were not subjected to biopsy, so that our histopathological validation was not independent of PET results. However, given the high co-incidence between the distribution patterns of ^{68}Ga-PSMA-HBED-CC and ^{18}F-DCFPyL (including sub-threshold correlates of ^{18}F-DCFPyL-positive lesions in the corresponding ^{68}Ga-PSMA-HBED-CC scan) as well as the morphological correlates of PET-positive lesions in the corresponding CT scan, there is accumulating evidence for the validity of ^{18}F-DCFPyL. We further note that four recent studies reported that PSMA-positive lesions could be histologically confirmed in 82%, 94%, 99% and 100% of cases, respectively (*13-16*).

Despite its limitations, the major strengths of this study are that we provide the largest cohort of BCR patients examined with ^{18}F-DCFPyL to date and that both tracers

were employed simultaneously at the same institution and analyzed by the same readers. Furthermore, we used a wide spectrum of mathematical models, including iterative Gleason-matched-pair analyses, to separately analyze prostatectomy and radiotherapy cohorts. Additionally, we applied variable PSA thresholds without making any a-priori assumptions. Finally, we cross-validated our results using a separate cohort of 25 patients who were sequentially examined with both tracers, thus allowing a direct comparison of the distribution patterns of ^{18}F- and ^{68}Ga-labeled PSMA tracers.

CONCLUSION

Our data suggest that ^{18}F-DCFPyL is non-inferior to ^{68}Ga-PSMA-HBED-CC while offering all the advantages of ^{18}F-labeling. Imaging with ^{18}F-DCFPyL may even exhibit improved sensitivity, when PSA levels are moderately increased to between 0.5µg/L and 3.5µg/L after prostatectomy. This is of high clinical relevance because within this PSA range PSMA-PET imaging detected most relapses at a limited stage. Hence, our findings provide a promising basis for validation of ^{18}F-DCFPyL in future prospective trials.

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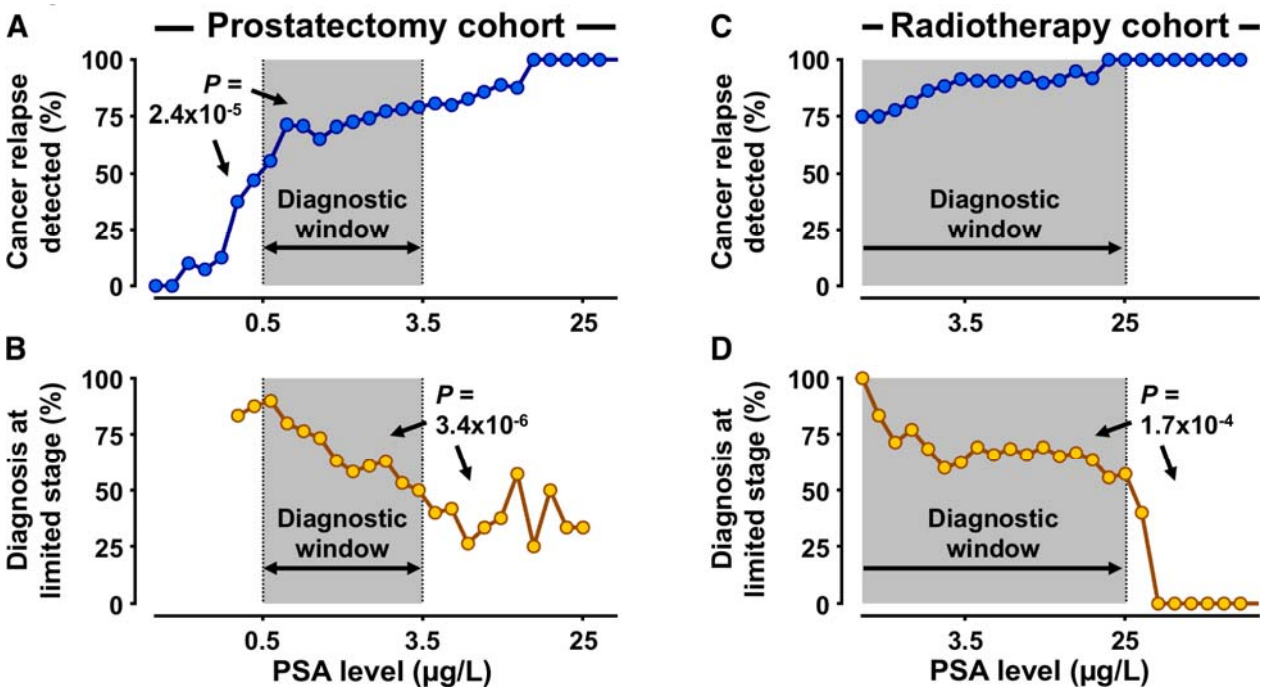


FIGURE 1. PSA-based stratification reveals an optimized PSA range for PET imaging. Patients were sorted by log-transformed PSA levels (x-axis) in ascending order. The fraction of PSMA-positive scans (blue) is plotted against PSA levels in prostatectomy (A) and radiotherapy (C) patients (*PSA sensitivity curve*). Similarly, the fraction of scans, displaying recurrent tumors at limited stage (orange) is plotted against PSA-levels in prostatectomy (B) and radiotherapy (D) patients. Based on these curves, a *diagnostic window* (gray) for PSMA-PET imaging was derived.

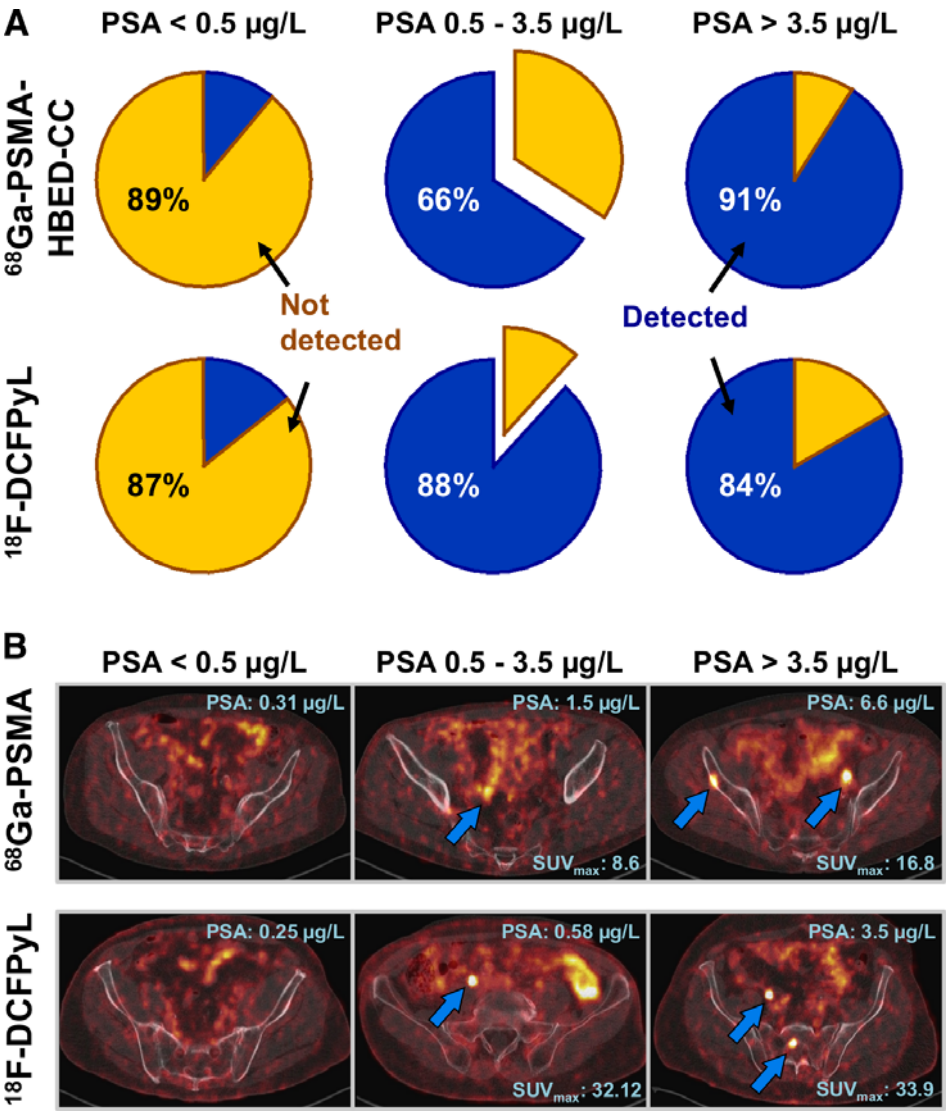


FIGURE 2. ^{18}F-DCFPyL displays enhanced sensitivity for localization of relapsed tumors after prostatectomy at limited stage. (A) Prostatectomy patients with BCR were examined with ^{68}Ga-PSMA-HBED-CC (top) or ^{18}F-DCFPyL (bottom). Pie charts display the fractions of PET-positive (blue) and PET-negative (orange) patients with PSA <0.5µg/L (left), >3.5µg/L (right) or 0.5-3.5µg/L (middle). (B) Representative PSMA-PET/CT images (fusion ratio 1:1), acquired with ^{68}Ga-PSMA-HBED-CC (top) or ^{18}F-DCFPyL (bottom). PSA level and SUV_{max} over the PSMA-positive lesion (blue arrows) are annotated for each scan.

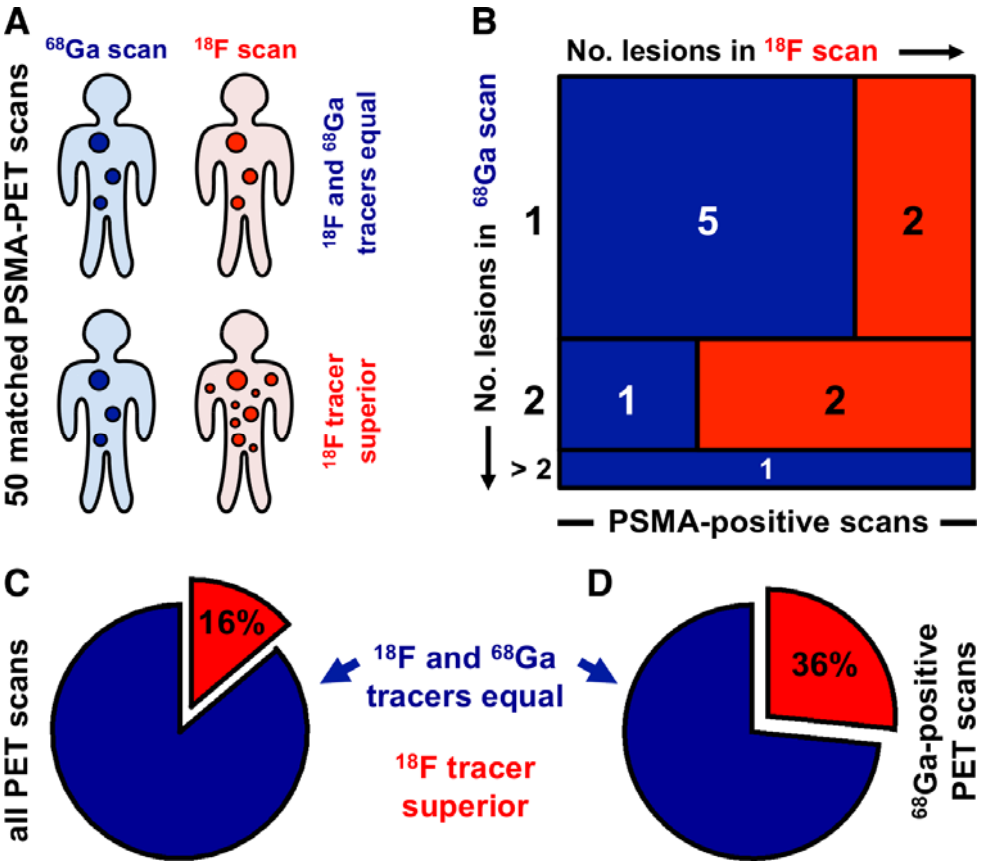


FIGURE 3. Direct comparison of the distribution patterns of ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC. (A) For 25 patients, sequentially examined with ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC, we determined whether the two tracers displayed an equal number of lesions. PSMA-negativity was consistent for ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC in all 14 PSMA-negative cases. (B) The mosaic plot compares PET-positivity scores between the two tracers in all 11 PSMA-positive cases (rows: PET-positivity of the ^{68}Ga scan, columns: PET-positivity of the corresponding ^{68}Ga scan). Each group is represented by a rectangle (red: more ^{18}F-DCFPyL-positive lesions; blue: equal PET-positivity scores). Rectangle areas reflect group sizes. (C,D) Pie charts display the fraction of patients with superior PET-positivity of the ^{18}F-DCFPyL scan (red) (all PET scans, 25 patients, C). Alternatively, only patients with positive ^{68}Ga scans were included (^{68}Ga-positive PET scans, 11 patients, D).

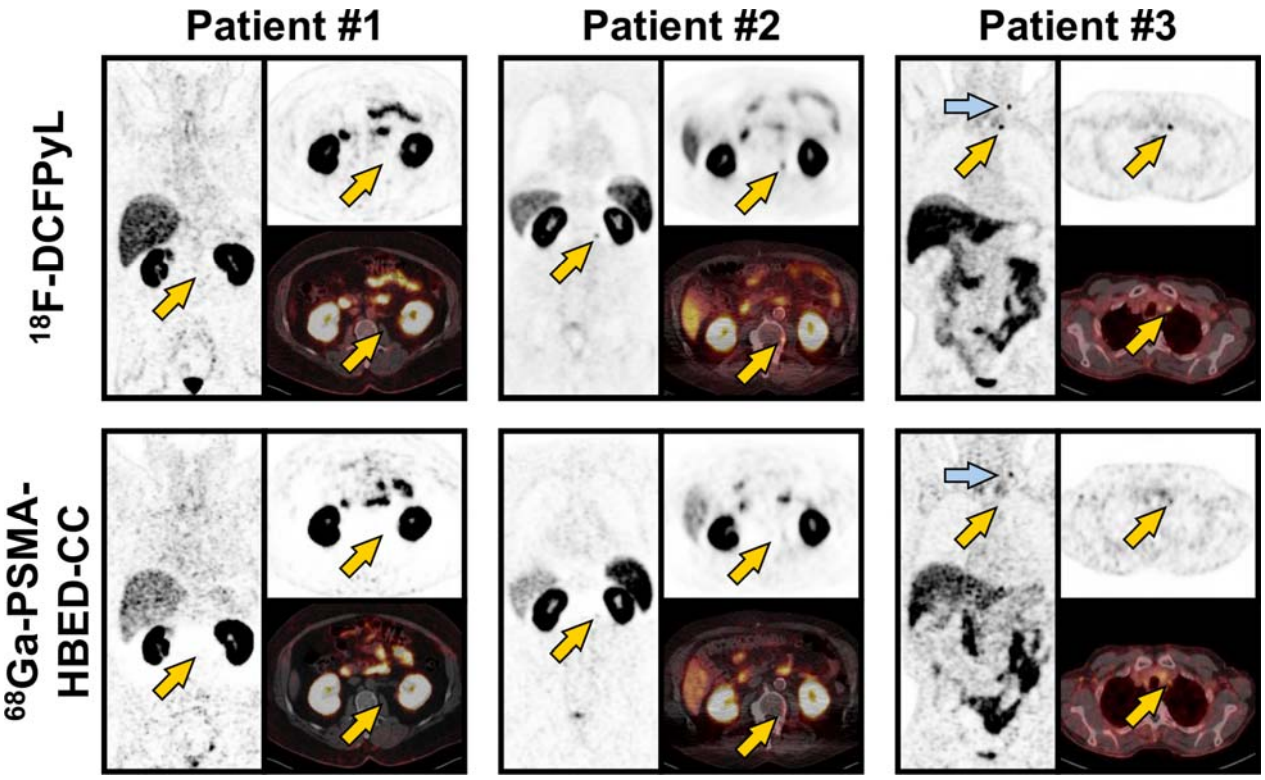
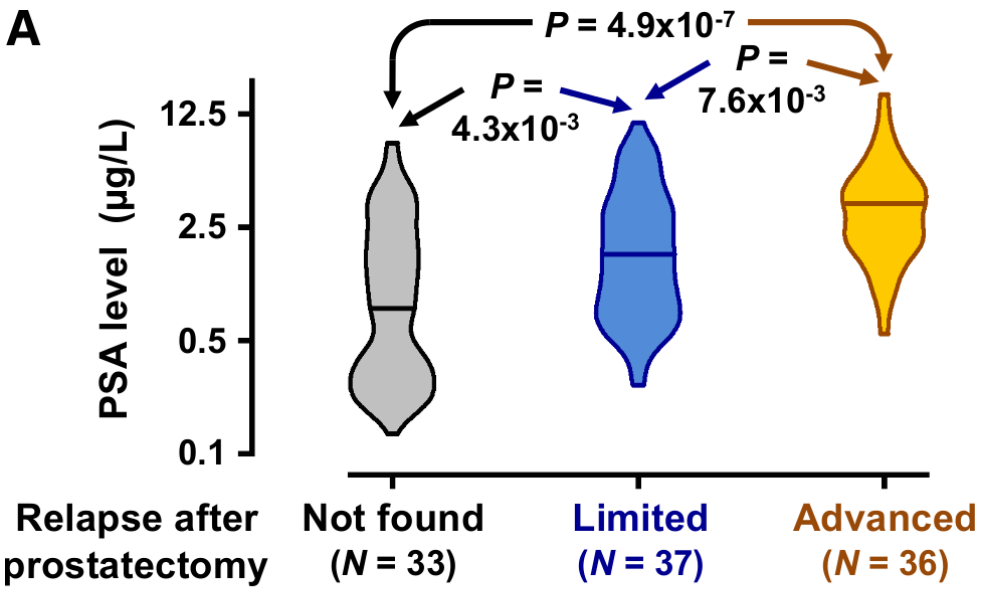


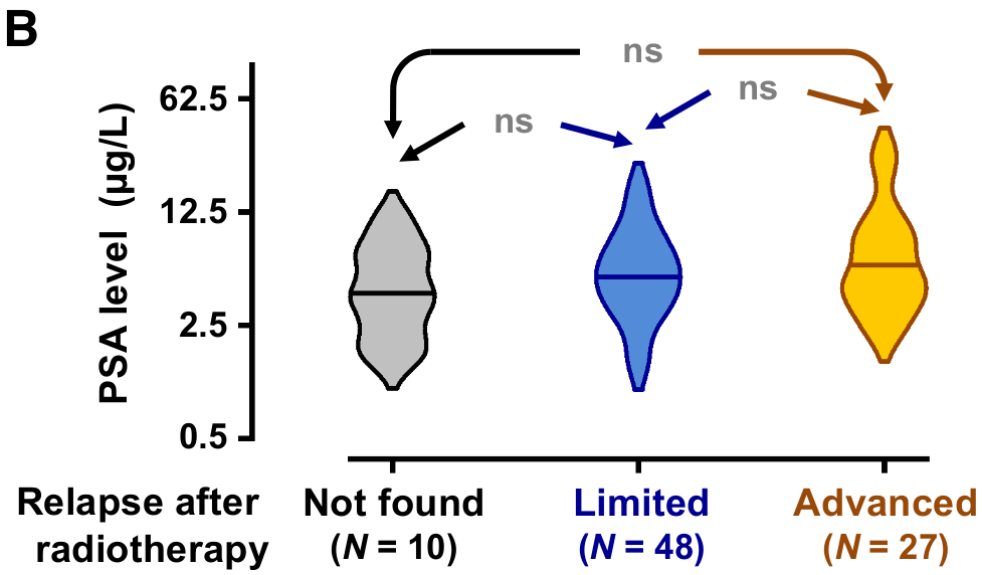
FIGURE 4. Representative matched PET scans. Images are shown for 3 PSMA-positive patients who were examined with ^{18}F-DCFPyL (top) and ^{68}Ga-PSMA-HBED-CC (bottom). While patients #1 and #2 displayed a clear ^{68}Ga signal extinction artifact, i.e. low activity counts between the kidneys, patient #3 displayed diminished tracer contrast on the ^{68}Ga scan. Coronary (left) and transversal (top right) slices are shown for each PET scan. Additionally, a PET/CT fusion image is displayed (bottom right). Arrows highlight differences between ^{18}F and ^{68}Ga scans. The same technical parameters (SUV windows, brightness, contrast, etc.) were employed for each corresponding image pair.

SUPPLEMENTAL FIGURE CAPTIONS

		PSA level			
		< 0.5 µg/L	0.5 - 3.5 µg/L	> 3.5 µg/L	
^{18}F-DCFPyL	pos.	1	15	11	19
	neg.	7	2	2	5
^{68}Ga-PSMA	pos.	1	23	22	56
	neg.	8	12	2	5
		prostatectomy			radio-therapy

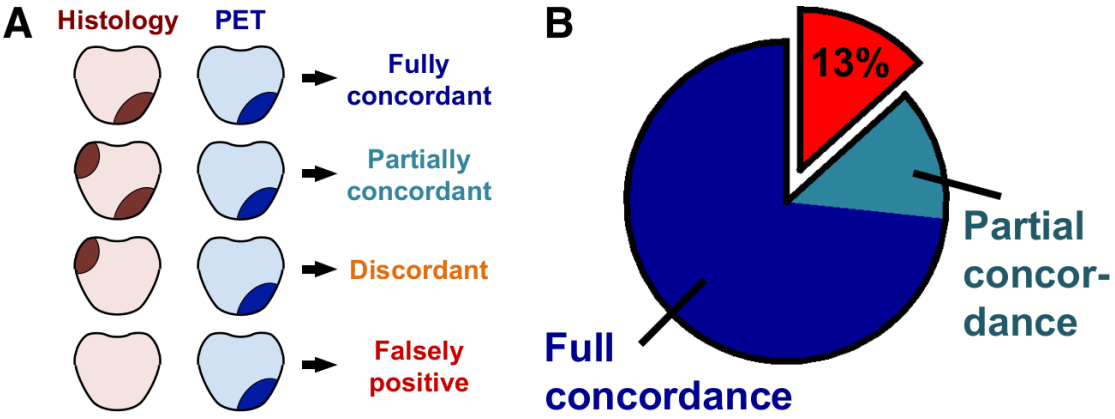
SUPPLEMENTAL TABLE 1. Absolute numbers of relapsed patients detected with PSMA-PET imaging. The table displays the absolute number of PET-positive (pos.) and PET-negative (neg.) scans acquired with either ^{18}F-DCFPyL (top) or ^{68}Ga-PSMA-HBED-CC (bottom, ^{68}Ga-PSMA). BCR patients received either surgery (left) or radiotherapy (right) as their initial therapy. For prostatectomy patients (left), numbers are shown separately for PSA levels <0.5µg/L (left), PSA levels >3.5µg/L (right) and PSA values within the diagnostic window (0.5-3.5µg/L, middle).



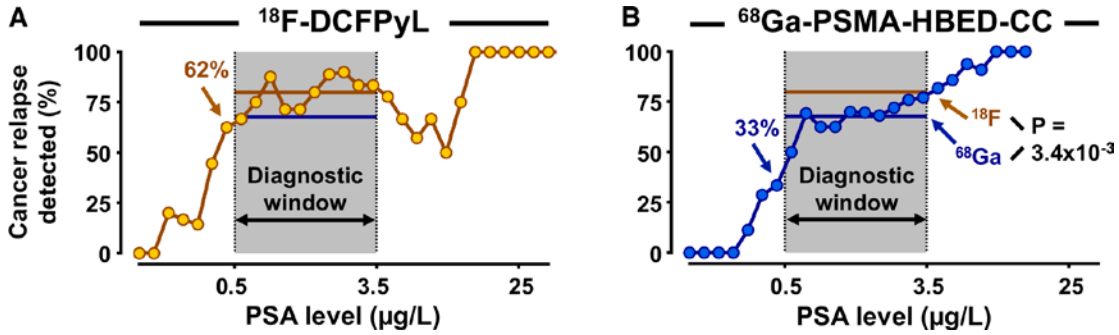


SUPPLEMENTAL FIGURE 1. PSA levels differ significantly at different stages of prostate cancer relapse after surgery. PET scans were split into three groups for patients after prostatectomy (*N*=106, A) and radiotherapy (*N*=85, B). The first group contained all scans, which did not display any PSMA-positive lesions (*not found*, gray). Images, which displayed tumor relapse, were subdivided into scans

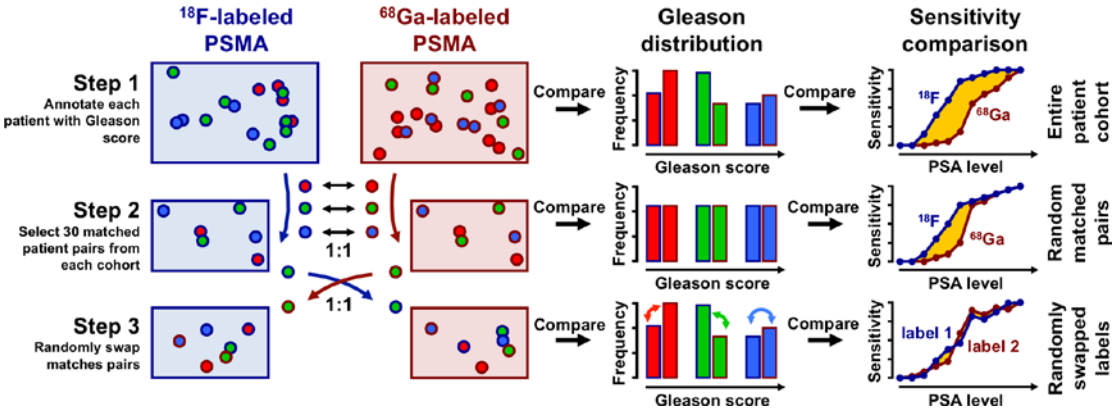
with a recurrent tumor at limited stage (*limited*, blue), i.e. local recurrence or infiltration into locoregional lymph nodes, and scans which displayed a recurrent tumor at an advanced stage (*advanced*, orange). Violin plots display the distribution of log-transformed PSA levels (y-axis) for each of these groups (kernel density estimation (KDE), using the probability density function of the normal distribution). Group medians are indicated by vertical bars. Significance values were calculated by two-tailed unpaired heteroscedastic t-tests.



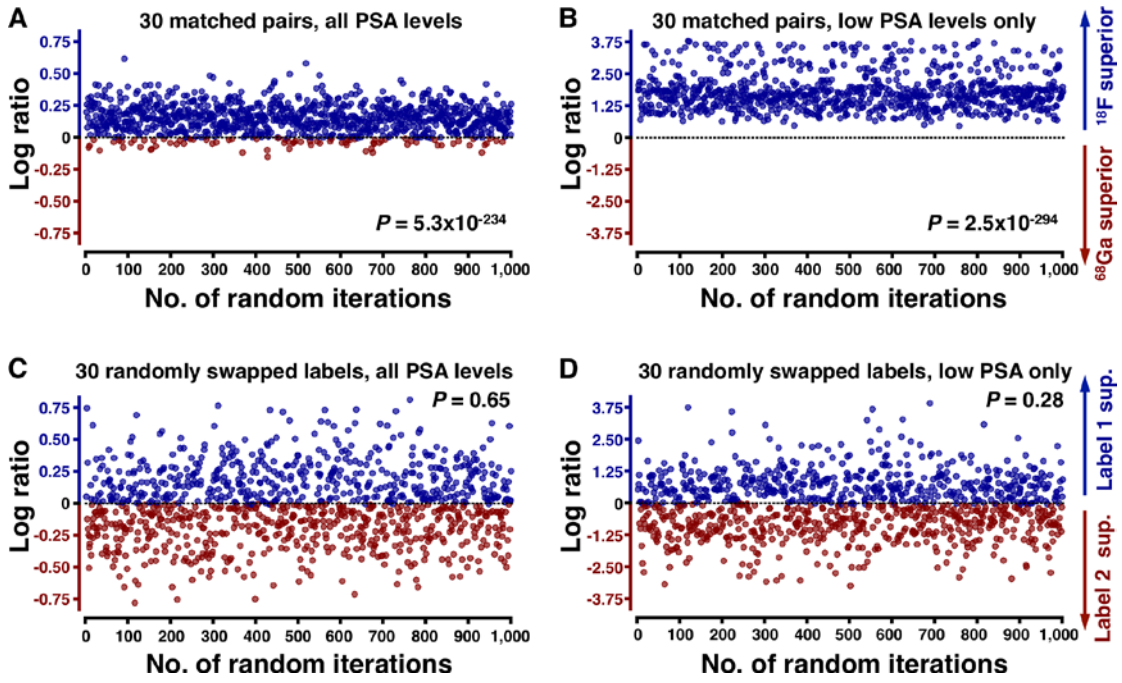
SUPPLEMENTAL FIGURE 2. Histological confirmation of PET-positive lesions. (A) We systematically examined tumor infiltration in the prostate fossa, based on 12 biopsies per patient. Based on these histology results, we compared for each segment tumor infiltration (red, left) with PSMA-positivity in in the corresponding PET scan (blue, right). That way, we differentiated between four different patterns of concordance / discordance. (B) The pie chart displays the fractions of fully (blue) and partially (cyan) concordant cases. Further, falsely positive cases are shown in red, for which PSA-positive lesions lacked histological confirmation.



SUPPLEMENTAL FIGURE 3. A PSA-stratified comparison of tracer sensitivity between ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC. In analogy to the sensitivity curve shown in Figure 1A for prostatectomy patients, PSA-stratified sensitivity curves were plotted separately for prostatectomy patients, examined with ^{18}F-DCFPyL (A, orange) or ^{68}Ga-PSMA-HBED-CC (B, blue), respectively. This analysis revealed discrete but robust differences between both tracer sensitivity curves. The *diagnostic window*, derived from Figures 1A and 1B, is plotted in gray. Arrows indicate point-sensitivity rates for PSA levels ranging around 0.45 µg/L (33% vs. 62%). Vertical lines display curve averages between 0.5 and 3.5 µg/L for ^{68}Ga-PSMA-HBED-CC (blue) and ^{18}F-DCFPyL (orange). Curve averages were compared by two-tailed t-tests.



SUPPLEMENTAL FIGURE 4. A Gleason-matched pair analysis of the sensitivity difference between ^{18}F- and ^{68}Ga-labeled PSMA tracers. (A) Schematic representation of the three steps of our Gleason-matched pair analysis. Step 1: In order to correct our comparison between ^{18}F- (blue, left) and ^{68}Ga-labeled (red, right) PSMA tracers for Gleason scores as potential confounders, we first annotated each patient (schematically represented as a dot) with his Gleason score (marked by different colors). Step 2: Secondly, we picked 30 random pairs of patients, from both the ^{18}F and the ^{68}Ga cohort. Each of these patient pairs was chosen with same Gleason score (matched pairs, annotated as 1:1). That way, we obtained subgroups of the ^{18}F and ^{68}Ga cohorts, each containing 30 patients with equal Gleason scores. In parallel to the analyses shown in Figure 1 and Supplemental Figure 3, we plotted cumulative tracer sensitivity curves for both subgroups and compared the PSA-stratified sensitivity. Step 3: Finally, we randomly exchanged matched patient pairs (annotated as 1:1) between the ^{18}F-DCFPyL and ^{68}Ga-PSMA-HBED-CC cohorts. In parallel to step 2, we compared sensitivity between subgroups of both tracer cohorts, which served as a negative control for our confounder correction analysis.



SUPPLEMENTAL FIGURE 5. Correction for Gleason scores preserves sensitivity difference between ^{18}F- and ^{68}Ga-labeled PSMA tracers. (A,B) We performed 1,000 random iterations, in order to derive 1,000 Gleason-matched groups of 30 patients, examined with ^{18}F-DCFPyL or ^{68}Ga-PSMA-HBED-CC, respectively. For each iteration, we calculated the log-transformed ratio between the average sensitivity in the ^{18}F and ^{68}Ga subgroups, respectively. Both of these subgroups shared the same Gleason scores. Ratios are plotted for each iteration, either for the entire range of PSA levels (A) or for PSA values below 1 µg/L (B). Iterations, in which sensitivity of ^{18}F-DCFPyL was superior, are colored in blue (ratio positive), whereas iterations in which ^{68}Ga-PSMA-HBED-CC displayed higher sensitivity are colored in red (ratio negative). The null hypothesis, assuming equal sensitivity between both tracers, is indicated as a dashed line. Significance was derived by comparing the distribution pattern of log-transformed ratios against the null hypothesis.