Results from extended lymphadenectomies with $[^{111}\text{In}]$PSMA-617 for intraoperative detection of PSMA-PET/CT-positive nodal metastatic prostate cancer

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Abstract

**Purpose:** Identification of suspicious PSMA-PET/CT-positive lymph node (LN) metastases (LNM) from prostate cancer (PCa) during lymphadenectomy (LA) is challenging. We evaluated an $^{111}$In-labelled PSMA ligand (DKFZ-617, referred to as $[^{111}\text{In}]$PSMA-617) as a $\gamma$-emitting tracer for intraoperative $\gamma$-probe application for resected tissue samples in PCa patients. Forty-eight hours prior to LA, $[^{111}\text{In}]$PSMA-617 was administered intravenously in 23 patients with suspected LNM on PSMA-PET/CT ($n = 21$ with biochemical relapse, $n = 2$ at primary therapy). Resected tissue samples (LN, LNM and fibrofatty tissue) were measured ex situ by a $\gamma$-probe expressed as counts per second (CPS norm). $[^{111}\text{In}]$PSMA-617 tissue sample uptake was measured by a germanium detector for verification and calculated as %IA lbm, (percent injected activity per kilogram lean body mass at time of surgery). Based on a clinical requirement for a specificity > 95%, thresholds for both ex situ measurements were chosen accordingly. Correlation of the results from PET/CT, $\gamma$-probe and germanium detector with histopathology was done.

**Results:** Eight hundred sixty-four LNs (197 LNM) were removed from 275 subregions in 23 patients, on average 8.6 ± 14.9 LNM per patient. One hundred four of 275 tissue samples showed cancer. Median $\gamma$-probe and germanium detector results were significantly different between tumour-affected (33.5 CPS norm, 0.71 %IA lbm) and tumour-free subregions (3.0 CPS norm, 0.03 %IA lbm) (each $p$ value < 0.0001). For the chosen $\gamma$-probe cut-off (CPS norm > 23) and germanium detector cut-off (%IA lbm > 0.27), 64 and 74 true-positive and 158 true-negative samples for both measurements were identified. Thirty-nine and 30 false-negative and 6 and 5 false-positive tissue samples were identified by $\gamma$-probe and germanium detector measurements.

**Conclusion:** $[^{111}\text{In}]$PSMA-617 application for LA is feasible in terms of an intraoperative real-time measurement with a $\gamma$-probe for detection of tumour-affected tissue samples. $\gamma$-probe results can be confirmed by precise germanium detector measurements and were significantly different between tumour-affected and tumour-free samples.

**Keywords:** Radio-guided surgery, $[^{111}\text{In}]$PSMA, Lymphadenectomy, Prostate cancer, Salvage lymph node dissection

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Background
Prostate cancer (PCa) is the most commonly diagnosed cancer in men. It can be cured by surgical removal of the prostate (radical prostatectomy) with pelvic lymphadenectomy (LA) or by radiotherapy (RT) [1–4]. The risk of metastases is mainly determined by the Gleason score, the level of PSA (prostate-specific antigen) and tumour extension (TNM status) [3, 4]. Pelvic and retroperitoneal lymph nodes (LN) are the first sites for metastases [2–4]. LN metastases (LNM), if assumed at primary therapy, should be removed at radical prostatectomy with LA in order to improve oncological outcome and enable correct staging [2–5]. An accurate preoperative detection of LNM by, e.g., positron emission tomography/computed tomography (PET/CT) is a prerequisite for successful surgery [6, 7].

Despite primary therapy, about 15–30% of the patients will develop a biochemical recurrence with elevated PSA level and clinical recurrence (metastases) possible at different sites (e.g. local relapse, LNM, bone). A systemic therapy such as ADT or chemotherapy is still the standard treatment in the stage of metastatic prostate cancer [3]. Regrettfully, during systemic therapy, PCa will inevitably develop a resistance to androgen deprivation or chemotherapy, which will end up in tumour progression again.

PET/CT targeting prostate-specific membrane antigen (PSMA) has demonstrated an excellent ability to detect LNM prior to surgery [6, 8–11] and is widely used as a tool for staging before primary therapy restaging of PCa patients in the setting of biochemical relapse [8–10, 12].

The most widely studied is 68Ga labelled to the small molecular inhibitor PSMA-11 via the HBED chelator ([68Ga]PSMA-11, also known as [68Ga]PSMA-HBED-CC) [13]. A recent meta-analysis published by Perera et al. showed on a per lymph node analysis a high sensitivity of 75% and a 99% specificity [14] for [68Ga]PSMA-11 PET/CT. Because of several advantages of the positron emitter 18F, 18F-labelled PSMA ligands ([18F]PSMA-1007, [18F]DCFPYL) were used more and more for imaging instead of 68Ga-labelled PSMA ligands [15, 16].

If PET/CT indicates “regional pelvic LNM” as the only finding at clinical recurrence, surgical removal (i.e. “salvage lymphadenectomy” (salvage-LA [17, 18])) of the lymphatic tissue or targeted RT may be suggested in patients in good general condition [6, 18, 19]. These “active approaches” are offered in order to delay systemic therapies [3, 18, 20, 21]. Against the background of the guidelines (e.g. European guidelines), salvage LND or target RT should be considered to be experimental and individual therapeutic approaches [3].

Locating suspected recurrent LNM during surgery is often very challenging in case of small LNM and reduced accessibility to the LNM (e.g. because of atypical location of LNM and tissue adhesions) [11]. Positron-emitting tracers are highly suitable for PET imaging but inappropriate for locating LNM during surgery because the γ-energy of their annihilation photons is too high for standard γ-probe devices. More appropriate for tracking of radioactively tagged LNM during surgery are γ-emitting tracers such as [111In]PSMA-I&IT [22], [99mTc]PSMA-I&S [23] or [111In]PSMA-DKFZ-617 [24] which also allow imaging as the PET tracers but with reduced spatial resolution, sensitivity and contrast due to the single photon emission computer tomography (SPECT) imaging technique [11, 25–27]. Without any doubt, there is a clear need to improve the identification of LNM during difficult surgery, regardless if at primary LA or at salvage LA [6, 11, 26, 28].

111In-labelled PSMA ligands were successfully introduced recently for imaging as well as for intraoperative use (radio-guided surgery (RGS)) [11, 21, 28–30]. After intravenous tracer injection prior to surgery, suspected LNM could be identified during surgery by applying a γ-probe with acoustic feedback. Accordingly, the surgeon is able to conduct in situ and ex situ measurement of suspected regions for LNM and resected tissue samples.

The recent development in the field of RGS for nodal recurrent PCa relapse is the application of 99mTc-labelled PSMA by Maurer et al. [25]. Based on the investigation of 31 patients with nodal PCa recurrence undergoing a salvage LND with the use of a γ-probe, they could show (specimen based) an impressive sensitivity of 83.6%, a specificity of 100% and an accuracy of 93% [25]. However, the identification of an optimal tracer for RGS salvage LND is still under investigation [26], and RGS is applied differently at the institutions offering this kind of experimental approach [11, 26, 31].

Recently, our group investigated the tracer uptake of [111In]PSMA-617 at a “single LN level” (manual separation of the resected tissue samples into single LN and LNM) in six patients demonstrating an excellent performance for the distinction between affected and non-affected LN (92.1% sensitivity, 98.9% specificity) [11].

The aim of this report was to evaluate the performance of the [111In]PSMA-617 on “region level” for a larger number of cases by analysing resected tissue samples from LA in order to distinguish between affected (mainly LNM) and non-affected tissue in men with suspected LNM on a PSMA-PET/CT using [68Ga]PSMA-11. Counts per second measured by γ-probe (ex situ), tissue sample tracer uptake measured with a germanium detector (ex situ) and PET/CT findings were correlated with histopathology. Based on the clinical requirement for high specificity (e.g. > 95%), the thresholds for both ex situ measurements were selected accordingly.
Material and methods

Patients

Between May 2015 and October 2016, 23 patients with the suspicion of exclusive LNM (without detectable bone or visceral metastases) on PSMA-PET/CT underwent LA guided by [111In]PSMA-617. Two of 23 patients underwent extended LA in the primary setting (radical prostatectomy), and 21/23 patients at the stage of biochemical recurrence (PSA > 0.2 ng/ml after radical prostatectomy) underwent salvage LA on a compassionate use basis. The local ethics committee approved this retrospective data analysis (no. 562/15). Informed consent was obtained from each subject, and all procedures were performed in accordance with the Helsinki Declaration. After surgery, the patients were treated according to the German S3 guidelines for treatment of PCa [2]; in case of PCa progression, a restaging by imaging (preferable PSMA-PET/CT) was done.

Tissue samples from eight subregions were excluded from the contingency analysis because a follow-up PSMA-PET/CT clearly showed the persistence or progression of the PET-positive lesion(s) and thereby that the LN(s) had obviously not been removed during surgery (e.g. difficulty in surgical access or because of inappropriate high surgical risks). Consistently, tissue samples from these “true-PET-positive” subregions have been therefore excluded from the analysis due to absence of tumour tissue and correspondingly negative γ-probe and germanium detector measurements.

Mean PSA follow-up was 31.2 ± SD 12.8 months; median follow-up at latest restaging (imaging) was 25.7 ± SD 16.3 months.

PSMA-HBED-CC-PET/CT and imaging analysis

[68Ga]PSMA-11 was conducted as described by Jilg et al. [6, 11]. Imaging was done 1 h after injection of averaged 202 ± SD 25 MBq [68Ga]PSMA-11. Contrast-enhanced diagnostic CT was used for anatomical correlation and PET attenuation correction. Two experienced nuclear medicine physicians evaluated all the PET/CT studies in consensus by side-by-side review of the co-registered PET and CT datasets using predefined PET window settings (inverted grey scale, SUV range 0 to 5 g/ml). Consistently, tissue samples from these “true-PET-positive” subregions have been therefore excluded from the analysis due to absence of tumour tissue and correspondingly negative γ-probe and germanium detector measurements.

Finally, the number of counts from the γ-probe used intraoperatively (ex situ measurement) gave the surgeon feedback if the suspected tumour tissue was resected or if the LA in this subregion had to be continued which ultimately increased the intensity of LA in this subregion.

Analysis with γ-probe

After removal of the 275 specimens, counts per second (CPS) were registered with a γ-probe (Neoprobe® GDS ex situ). To generate comparable data between patients, CPS were normalised (CPS\text{norm}) to the injected activity per kilogram lean body mass and decay-corrected to the time of surgery (48 h representing the median time after injection of [111In]PSMA-617 and the median time of ex situ γ-probe measurements) in the patient group.

Analysis with germanium detector

All samples were weighed. Tissue sample activity measurements were done with a high-purity germanium detector (Canberra Inc., model GX2018-CP5+, calibrated with a multi-isotope reference source, type VZ-2139/
Table 1 Patient characteristics, history of prostate cancer and outcome from lymphadenectomies from 23 patients undergoing lymphadenectomy

| Parameters | Values |
|------------|--------|
| **iPSA at primary therapy (ng/ml)** | Mean ± SD/median/range |
| | 10.79 ± 7.5/8.8/3.37–37.0 |
| **Primary therapy, n** | |
| Radical prostatectomy | 21/23 (91.3%) |
| Radiotherapy | 2/23 (8.7%) |
| **Gleason score, n** | |
| 7a | 4 (17%) |
| 7b | 7 (31%) |
| 8 | 5 (22%) |
| 9 | 7 (30%) |
| **111In-PSMA-guided LA overall, n** | |
| Primary | 2/23 (8.7%) |
| Salvage lymph node dissection | 21/23 (91.3%) |
| **Age at lymphadenectomy (years)** | Mean ± SD/median/range |
| | 67.5 ± 6.6/67/52–78 |
| **PSA at 111In-PSMA-guided LA (ng/ml)** | Mean ± SD/median/range |
| | 7.9 ± 12.9/1.8/0.03–56.2 |
| **Time between PET/CT and 111In-PSMA-guided LA (months)** | Mean ± SD/median/range |
| | 3.2 ± 1.6/3.0/1.0–8.0 |
| **Time between primary therapy and 111In-PSMA-guided LA (years) (n = 21)** | Mean ± SD/median/range |
| | 4.9 ± 3.7/4.1/1.5–13.7 |
| **Histological outcome for 23 patients, n** | |
| LA with positive histology | 21/23 (91.3%) |
| LA with negative histology | 2/23 (8.7%) |
| **Topography of 111In-PSMA-guided LA in 23 patients, n** | |
| Pelvic right and left | 13/23 (57%) |
| Pelvic left only | 2/23 (9%) |
| Pelvic right only | 1/23 (4%) |
| Pelvic right and left and retroperitoneal | 7/23 (30%) |
| **Topography of subregions with confirmed PCa, n** | |
| Pelvic left, n | 38/275 (37%) |
| Pelvic right, n | 41/275 (40%) |
| Retroperitoneal | 24/275 (23%) |
| **Histological outcome for 275 subregions with 275 samples, n** | |
| Subregions/samples with LNM | 104/275 (37.5%) |
| Subregions/samples without LNM | 171/275 (62.5%) |
| Subregions/samples with additional non-nodal PCa-tissue | 7/104 (5.8%) |
| **Number of LN removed (n)** | |
| Overall | 864 |
| Per patient (mean ± SD/median/range) | 37.6 ± 17.2/38.0/2.0–82.0 |
| **Number of LNM removed (n)** | |
| Overall | 197 |
| Per patient (mean ± SD/median/range) | 8.6 ± 14.9/4.0/0.0–71.0 |
| **LNM fraction per patient (LNM×100/LN = %)** | Mean ± SD/median/range |
| | 20.9 ± 24.4/12.5/0.0–87.1 |

LNM lymph node metastases, LN lymph node, PSA prostate-specific antigen, LA lymphadenectomy
NG3 from Eckert&Ziegler Nuclitec’s DKD-accredited measurement laboratory in Germany, and cross-calibrated for tissue sample geometry. Tracer uptake was calculated as percent injected activity per kilogram lean body mass, corrected for decay:

\[
\%\text{IA}_{\text{lbm}} = \frac{\text{tissue sample activity} [\text{Bq}] \cdot 100}{(\text{injected activity} [\text{Bq}]/2^{-(\Delta t/T_{1/2})})/\text{lean body mass} [\text{kg}]}
\]

with \(\Delta t\) and \(T_{1/2}\) being the delay between patient injection and sample measurement and the half-life of \(^{111}\text{In}\) (2.81 days [32]), respectively. Lean body mass was calculated according to Janmahasatian et al. [33] and used for normalisation instead of body weight, since fat tissue does not participate in normal tracer distribution.

**Histopathological analysis**

All resected LNs (i.e. the entire LN in case of small LNs, one central slice in case of LNs > 4 mm) were fixed with formalin and embedded in paraffin. The pathologist was not aware of the PET findings and did not know the clinical report of the tissue from the surgeon. Histopathologic evaluation was performed by one pathologist on haematoxylin and eosin (H&E)-stained tissue slides.

**Statistical analysis**

Analysis of findings (positive, negative) of PET/CT, γ-probe and germanium detector in a contingency table was done for 267 of 275 subregions; 8 subregions were excluded from the contingency analysis because a follow-up PSMA-PET/CT showed the persistence or progression of the PET-positive lesion(s) indicating that the LN(s) had not been removed during surgery. Descriptive statistics were done by calculating means, standard deviations (SD), medians and ranges. From all resected tissue samples, we had the information of the γ-probe and germanium detector results as well as the histopathological outcome. Based on these data, we performed a ROC curve analysis (receiver operating characteristic curve). Continuous variables were compared with a two-sided unpaired Mann-Whitney test. Diagnostic accuracy of γ-probe (CPS\text{norm}), germanium detector tracer-uptake measurement (%IA\text{lbm}) and PET/CT was described by a contingency table. We used Prism 6 GraphPad.

**Results**

Clinical data summarising the stage of PCa and LAs of the 23 patients are shown in Table 1. In the majority (21/23), LA were done in the setting of salvage LA because of suspected LNM on a PSMA-PET/CT at the stage of biochemical recurrence. Mean PSA level at surgery was 7.9 ng/ml. For 21 of 23 patients, histological outcome confirmed the presence of PCa in the removed tissue samples; in the remaining 2/23 patients, it was not possible to remove the PET-positive lesions. By PSMA-PET/CT follow-up, the PET-positive lesions in those two patients showed a clear progression or persistence of the metastases. Complications from surgery, analysed according to Clavien-Dindo [34], are shown in Table 2.

Figure 1a–f shows two representative PSMA-PET/CT and SPECT/CT with an LNM in the left parailiacal region (patient no. 1) and in the right parailiacal region (patient no. 2). A median of 38 LN had been removed during LA per patient, of which a median of 4.0 turned out to be metastases. Overall, a high number of subregions (\(n = 275\)) underwent LA, and a high number of LN (\(n = 864\)) had been removed (Table 1).

The workflow of the sample processing is shown in Fig. 2. From 275 subregions, 275 tissue samples, consisting of LN, LNM and fibrofatty tissue, were removed separately and measured with a γ-probe and in a germanium detector ex situ. The origin of 275 tissue samples is shown in Table 1.

Figure 3 shows representative nodal fibrofatty tissue samples from one subregion (a, b), γ-probe measurements (c) and sample vessels for the tracer uptake measurements (d).

Histopathological analysis of the 275 tissue samples yielded 171 samples free of tumour and 104 samples with tumour (mainly LNM).

Results of CPS\text{norm} and %IA\text{lbm} tracer uptake from tumour-containing (\(n = 104\)) and tumour-free (\(n = 171\)) samples were significantly different (both \(p < 0.0001\)) which is shown in Table 3. Samples without tumour had a median of 3.0 CPS\text{norm} and 0.03 %IA\text{lbm} compared with a median of 33.5 CPS\text{norm} and 0.71 %IA\text{lbm} measured from samples with tumour. This high degree of separation by γ-probe and germanium detector measurements is also shown in Fig. 4 c and d by comparing the medians ± range of CPS\text{norm} and %IA\text{lbm} in scatter plots. Each corresponding value of CPS\text{norm} and %IA\text{lbm} from 275 tissue samples is displayed in Fig. 4 a and b in a waterfall plot. Red lines highlight the cut-off(s) for both measurements.

Based on the clinical requirement of high specificity (> 95%), a threshold of > 23 CPS\text{norm} and > 0.27 %IA\text{lbm} was determined to reduce the risk of false-positive samples. For a cut-off of > 23 CPS\text{norm}, we determined a sensitivity of 62.1% and specificity of 96.3%. It has to be mentioned that for patient-individual γ-probe CPS measurements during surgery, the threshold of 23 CPS\text{norm} has to be multiplied by the injected activity per kilogram lean body mass. After applying the cut-off of > 0.27 %IA\text{lbm}, we calculated a sensitivity of 71.2% and a specificity of 96.9% comparable to PET/CT (sensitivity of 79.8% and specificity of 96.9%) (Table 4).
Figure 5 shows the intersections of true- and false-positive as well as true- and false-negative subregions from PSMA-PET/CT findings, γ-probe and germanium detector measurements with Venn diagrams.

One hundred forty-seven of 171 (85.9%) specimens free of cancer were correctly identified as true negative by all three modalities (Fig. 5c). In contrast, only 54/104 (51.9%) of the specimens with cancer had been identified correctly as true positive by all three modalities (Fig. 5a).

Additional file 1: Table S1 shows data about the clinical course after surgery regarding PSA values and follow-up imaging by mainly PSMA-PET/CT. Most (14/23) of the patients developed clinical progression after salvage LND.

**Discussion**

By targeting PSMA on cancer cells with $^{111}$In- or $^{99m}$Tc-labelled PSMA ligands, RGS was successfully introduced for intraoperative use with a γ-probe and acoustic feedback by Maurer et al. [11, 21, 25, 26, 28]. As those previous works analysed so-called "mixed-tissue samples" containing LN, LNM and fibrofatty tissue, a more specific investigation of the $^{111}$InPSMA-617 tracer uptake at a single LN level was done by our group [11]. Based on 275 tumour-free single LNs and 35 single LNM s, after manual separation out of resected tissue samples, a sensitivity of 92.1% and specificity of 98.9% were reported, indicating a convincing tumour-specific uptake in PCa lesions [11].

Rauscher et al. reported on 31 men undergoing RGS with $^{111}$In-labelled PSMA ligand because of nodal PCa relapse. One hundred forty-five mixed-tissue samples were resected (51 tumour bearing) and measured ex situ with a γ-probe. Sensitivity was 92.3%; specificity was 93.5% [29]. RGS described by Maurer et al. and Rauscher et al. was predominantly restricted to PET-positive regions only and their immediate vicinity [28, 29].

A unique feature in our study is the measurement of tracer uptake of the resected tissue samples directly after surgery in a germanium detector, which can be considered as a precise verification of γ-probe measurement. Such an effort is not a part of clinical routine and was done only for the cohort presented here. Due to tracer availability, isotope costs and radiation protection issues, $^{99m}$Tc-labelled PSMA is currently used for RGS, and our cohort provides uniform data from the relatively limited experience with RGS $^{111}$In-labelled PSMA.

Although in situ γ-probe measurement was done in this study, a usual standard γ-probe with “pencil-geometry” is not well engineered yet in terms of size and angle for the application in a narrow pre-treated pelvic region.

During practice in salvage LND with a γ-probe, it turned out that sufficient contact between the γ-probe and the tissue region (ideally 90° corresponding to the collimator angle) in situ was not always possible because of restricted spatial conditions. Therefore, we focused on the ex situ measurements of the removed tissue samples with the γ-probe (CPS$_{norm}$) and the subsequent tracer
uptake measurements with a germanium detector (%IA$_{bnm}$).

Usually, tissue samples from one subregion consist of LN, LNM, fat, small vessels and fibrofatty tissue. Because of unspecific tracer uptake within such a tissue mix, false-positive signals might arise at γ-probe and germanium detector measurements. On the other hand, a specific tracer signal in tumour tissue might be weakened due to attenuation in large mixed-tissue samples enclosing fibrofatty tissue, fat or small vessels, leading to false-negative results, especially in the case of a very small amount of tumour tissue.

Because of the clinical need for a low number of false-positive results (to prevent the surgeon from misinterpretation and early termination of the LA), expressed by a high specificity (e.g. > 95%), we chose
the threshold for both measurements appropriately. Consequently, both measurements yield a relatively low sensitivity (Table 4).

Figure 5a shows an overlap of 51.9% (54/104) for true-positive subregions for all three methods in the Venn diagram. With 83/104 true-positive-rated subregions, the detection rate of PET/CT seems to be excellent compared with the germanium detector (75/104) and γ-probe (64/104) measurement. This might result from the fact that the resected mixed-tissue samples (LN, fat, fibrofatty tissue) were larger when undergoing ex situ measurements by germanium detector and γ-probe than the spatial resolution of PET/CT. Under these circumstances, PET/CT is able to discriminate between lesions with specific PSMA uptake and the surrounding tissue with lower, unspecific uptake than the ex situ measurements.

Interestingly, there was no overlap for false-positive subregions between PET/CT, germanium detector and γ-probe (Fig. 5b). There is no obvious reason for this result but the methodology of the three measurements is very different and the uptake times between [⁶⁸Ga]PSMA-11 imaging and [¹¹¹In]PSMA-617 measurements differ too. While PSMA-PET/CT was recorded 1 h post injection, it might have a higher uptake in regions with higher perfusion. [¹¹¹In]PSMA-617 was measured 48 h post injection with nearly complete tracer wash-out but remaining unspecific tracer accumulation [27].

Germanium detector measurements are very accurate in determining activity in specified sample geometry. For larger samples containing mixed-tissue compositions with fat, fibrofatty tissue, small vessels and LN, the interpretation of the results can only be done as an averaged value for the whole sample. In addition to a generally possible lack of PSMA expression, false-negative results might be caused by very low tumour load (e.g. very small LN) and attenuation of tracer signal due to enclosed tissue in the sample (Fig. 5d). Measurements of mixed-tissue samples with a germanium detector as presented in this study are therefore not comparable to the known excellent sensitivity (92.3%) and specificity (93.5%) from

### Table 2 Complications arising from 23 lymphadenectomies

| Complications according to Clavien-Dindo | Grade | Overall, n (%) |
|------------------------------------------|-------|---------------|
| Lymphorrhea                              | I     | 3/23 (13.04%) |
| Transient weakness of hip flexor         | I     | 1/23 (4.35%)  |
| Wound dehiscence                         | I     | 1/23 (4.35%)  |
| Wound infection treated with antibiotics | II    | 1/23 (4.35%)  |
| Lymphorrhea requiring drainage, secondary| IIIa  | 2/23 (8.7%)   |
the analysis of “single lymph nodes” in such a device [11]. The reasons are the methodological limitations discussed.

As shown by our group previously [35], the LN detection rate for PSMA-PET/CT was reduced to 50% for tumour deposits < 2.2 mm in LNM; consequently, one could assume that the false-negative subregions might harbour very small amounts of tumour (only detectable at histopathology) or tumour cells were negative for PSMA.

However, currently, we could not identify a common pattern of subregions rated as false negative or false positive on PET/CT, γ-probe or at germanium detector analysis. Neither the clinical data from patients providing those subregions nor the locations of the subregions were apparently different from the rest.

The majority of our small cohort developed clinical recurrence (14/23) (follow-up was 31.2 ± SD 12.8 months). From our experience [17] and the latest literature (meta-analysis of 27 series), it is known that the 2- and 5-year biochemical progression-free survival rates range from 23 to 64% and from 6 to 31%, respectively; the 5-year overall survival is approximately 84% [18, 36]. By rough estimation, our cohort has an outcome which is not better than that of patients treated with conventional salvage LND.
Currently, salvage LND for patients with nodal recurrent LNM or nodal high-volume LNM at primary therapy (rad. PE) at our centre is done guided by $^{99}$mTc-labelled PSMA with ex situ $\gamma$-probe measurement but without germanium detector measurement in the laboratory, similar to the approach of Maurer et al. [25].

The clinical benefit of RGS salvage LND compared with conventional salvage LND is still unclear. Knipper et al. made the first approach of comparing conventional salvage LND ($n = 29$) versus salvage LND with radio-guided assistance ($n = 13$) [31]. After a short follow-up (6 weeks), they were able to show a better PSA decline in general for the RGS salvage LND compared with

![Fig. 5 a–d Venn diagrams illustrating the intersections between the results of PSMA-PET/CT, tracer uptake determined with the germanium detector and ex situ $\gamma$-probe measurements. Intersections for a true-positive, b false-negative, c true-negative and d false-positive subregions in 104 subregions with histologically proven metastases and 171 subregions free of metastases](image-url)

### Table 3 $\gamma$-probe and germanium detector measurements from 275 tissue samples of 275 subregions

|                    | Samples with tumour ($n = 104$) | Samples without tumour ($n = 171$) | Mann-Whitney test, $p$ value |
|--------------------|---------------------------------|-----------------------------------|-----------------------------|
| Germanium detector [% IA lbm]* | 0.71/2.6 ± 5.4/0.008–36.33 | 0.03/0.07 ± 0.17/0–0.817 | < 0.0001 |
| $\gamma$-probe [CPS norm]† | 33.5/49.0 ± 49.5/0–213.0 | 3.0/5.9 ± 11.2/0–118.0 | < 0.0001 |

*Percent injected activity per kilogram lean body mass
†Counts per second decay corrected to 48 h and normalised to injected activity megabecquerel per kilogram lean body mass
Table 4: Agreement between PSMA-PET/CT, γ-probe, germanium detector and histopathology in 267 subregions

| Subregions with tissue samples (n = 267) | Histopathology | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------------------------------------|----------------|----------------|----------------|---------|---------|
| PSMA-PET/CT findings†                  |                |                |                |         |         |
| Positive                                | 83             | 5              | 79.8           | 96.9    | 94.3    | 88.3    |
| Negative                                | 21             | 158            | 62.1           | 96.3    | 91.4    | 80.2    |
| γ-probe [CPS norm]§‡                    |                |                |                |         |         |
| Positive                                | 64             | 6              | 71.2           | 96.9    | 93.7    | 84.0    |
| Negative                                | 39             | 158            |                |         |         |
| Germanium detector [%IA lbm]†          |                |                |                |         |         |
| Positive                                | 74             | 5              |                |         |         |
| Negative                                | 30             | 158            |                |         |         |

Exclusion of 8/275 subregions because a follow-up PSMA-PET/CT showed the persistence or progression of the PET-positive lesion(s) indicating that the LN(s) had not been removed at surgery

Abbreviations: IA lbm: Percent injected activity per kilogram lean body mass; CPS: Counts per second; LA: Lymphadenectomy; LN: Lymph node; LNM: Lymph node metastasis; PCa: Prostate cancer; PET/CT: Positron emission tomography/computed tomography; PSA: Prostate-specific antigen; PSMA: Prostate-specific membrane antigen

**Limitations**

Although the number of patients (n = 23) seems to be relatively low, the actual sample size (n = 864 LN from 275 subregions) was very high. Even if the majority of the patients were at high risk for PCa, heterogeneity was present (PSA level, Gleason score, different kind of previous therapies).

Generally, there is a selection bias because only patients with suspected LNM on PSMA-PET/CT and therefore known PSMA-positive lesions were included in this study. Furthermore, ideally in all patients, an identical bilateral template LA should have been performed which is hard to realise for all patients for different reasons (e.g. surgical access). However in our cohort, only 4/23 (13%) received a unilateral lymphadenectomy (at the site of a PET-positive lesion); this might weaken the reliability of sensitivity and specificity in our calculation. However, our high number of analysed “subregions” (small anatomical regions) (n = 275) and our high number of removed LN (n = 864) might provide better diagnostic information than a simple summary in, e.g. left- or right-sided lymph nodes. In summary, 179 PET-negative and 88 PET-positive subregions underwent an LA, which allows, in our view, specificity and sensitivity to be calculated.

**Conclusions**

At a region-based analysis, the results for tracer uptake measurements (γ-probe, germanium detector) were significantly different between tumour-affected and tumour-free samples. In the clinical setting with the need for high specificity (> 95%) and a corresponding chosen threshold for γ-probe measurement (confirmed by precise germanium detector measurements), the use of [111In]PSMA-617 at LA is feasible for the distinction between affected and unaffected tissue samples and valuable for ex situ tumour verification during surgery.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s13550-020-0598-2.

Additional file 1: Table S1. PSA-response and clinical course after surgery.

**Abbreviations**

%IA lbm: Percent injected activity per kilogram lean body mass; CPS: Counts per second; LA: Lymphadenectomy; LN: Lymph node; LNM: Lymph node metastasis; PCa: Prostate cancer; PET/CT: Positron emission tomography/computed tomography; PSA: Prostate-specific antigen; PSMA: Prostate-specific membrane antigen

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**Authors’ contributions**

WSS and CAJ conducted the surgical treatment (lymphadenectomies). PTM, MM and CAJ designed the study. CAJ applied for ethical approval. KR and MvB were responsible for the patient recruitment and for the organisational procedures between the Department of Urology and Nuclear Medicine, gathered informed consent from the patients and supported the data analysis (data collection). CS and HCR were responsible for the interpretation of the PET/CT images. MB was responsible for the synthesis of [111In]labelled PSMA. VD investigated the resected tissue samples and generated the histopathological results. MM conducted analysis and interpretation of γ-probe measurement and the gamma detector measurement and revised the
The authors declare that they have no competing interests.

Authors’ information
Not applicable.

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All procedures performed in studies included in the current analyses were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The local ethics committee approved this retrospective data analysis (no. 562/15) (Ethik-Kommission der Albert-Ludwigs-Universität Freiburg, Vorsitzender Prof. Dr. R. Korinthenberg, Engelberger Straße 21 79106 Freiburg. Title of the vote: Eignung der nuklearmedizinischen Diagnostik beim Prostatakarzinom in verschiedenen klinischen Stadien. Positives Votum 562/15).

Informed consent was obtained from each subject.

Consent for publication
Not applicable.

Competing interests
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