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ORIGINAL ARTICLE

Diagnosis of recurrent prostate cancer with PET/CT imaging using the gastrin-releasing peptide receptor antagonist ^{68}Ga-RM2: Preliminary results in patients with negative or inconclusive [^{18}F]Fluoroethylcholine-PET/CT

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Abstract

Purpose/background [^{18}F]fluoroethylcholine (^{18}FECH) has been shown to be a valuable PET-tracer in recurrent prostate cancer (PCa), but still has limited accuracy. RM2 is a gastrin-releasing peptide receptor (GRPr) antagonist that binds to GRPr on PCa cells. Recent studies suggest that GRPr imaging with PET/CT is a promising technique for staging and restaging of PCa. We explore the value of GRPr-PET using the ^{68}Ga-labeled GRPr antagonist RM2 in a selected population of patients with biochemically recurrent PCa and a negative/inconclusive ^{18}FECH-PET/CT.

Material and methods In this retrospective study 16 men with biochemical PCa relapse and negative (n = 14) or inconclusive (n = 2) ^{18}FECH-PET/CT underwent whole-body ^{68}Ga-RM2-PET/CT. Mean time from ^{18}FECH-PET/CT to ^{68}Ga-RM2-PET/CT was 6.1 ± 6.8 months. Primary therapies in these patients were radical prostatectomy (n = 13; 81.3%) or radiotherapy (n = 3; 18.7%). 14/16 patients (87.5%) had already undergone salvage therapies because of biochemical relapse prior to ^{68}Ga-RM2-PET/CT imaging. Mean ± SD PSA at ^{68}Ga-RM2-PET/CT was 19.4 ± 53.5 ng/ml (range 1.06–226.4 ng/ml).

Results ^{68}Ga-RM2-PET/CT showed at least one region with focal pathological uptake in 10/16 patients (62.5%), being suggestive of local relapse (n = 4), lymph node metastases (LNM; n = 4), bone metastases (n = 1) and lung metastasis with hilar LNM (n = 1). Seven of ten positive ^{68}Ga-RM2 scans were positively confirmed by surgical resection and histology of the lesions (n = 2), by response to site-directed therapies (n = 2) or by further imaging (n = 3). Patients with a positive ^{68}Ga-RM2-scan showed a significantly higher median PSA (6.8 ng/ml, IQR 10.2 ng/ml) value than those with a negative scan (1.5 ng/ml, IQR 3.1 ng/ml; p = 0.016). Gleason scores or concomitant antihormonal therapy had no apparent impact on the detection of recurrent disease.

Conclusion Even in this highly selected population of patients with known biochemical recurrence but negative or inconclusive ^{18}FECH-PET/CT, a ^{68}Ga-RM2-PET/CT was helpful to localize PCa recurrence in the majority of the cases. Thus, ^{68}Ga-RM2-PET/CT deserves further investigation as a promising imaging modality for imaging PCa recurrence.

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Keywords ^{68}Ga-RM2 · PET/CT · Prostate cancer · Gastrin-releasing peptide receptor (GRPr) · Bombesin receptor

Introduction

Radical prostatectomy and radiotherapy (RT) are standard therapies for patients with primary prostate cancer (PCa) [1]. Despite good clinical response after primary therapy, PCa recurs in 20–50% of cases, depending on stage at diagnosis [1]. PCa recurrence can be reliably detected by an increase of the prostate specific antigen (PSA) in serum [1, 2]. Identification of the exact site of tumor recurrence (e.g., lymph node metastases or bone metastases) is critical for further decision-making. Unfortunately, to date, none of the conventional imaging modalities such as transrectal ultrasound, computed tomography (CT), magnetic resonance imaging (MRI) or bone-scintigraphy provides a sufficient diagnostic accuracy for reliable detection of primary or recurrent PCa [2, 3] in patients with low PSA levels who are most likely to benefit from local therapies. Therefore, there is a need for better imaging modalities for staging and re-staging of PCa and treatment monitoring.

Positron emission tomography (PET) with ^{11}C- or ^{18}F–labeled choline derivatives is superior to MRI and CT for recurrent PCa [4, 5] but the sensitivity of choline-PET/CT for detecting sites of tumor strongly depends on the PSA (Prostate specific antigen) value at the time of imaging, Gleason score, and other clinical factors [4]. Therefore, there is a clear need for other imaging agents to localize recurrent prostate cancer. Most attention has been paid to tracers targeting the prostate-specific membrane antigen (PSMA), the androgen receptor and the gastrin-releasing peptide receptor (GRPr) (for review see [6]).

The GRPr (also known as bombesin receptor) and its ligand, the gastrin-releasing peptide (GRP), are physiologically expressed in the central nervous system and the gastrointestinal tract [7]. An overexpression of GRPr has been observed in a variety of malignant tumors but most consistently in PCa and its percursors [7]. An autoradiographic study found markedly increased binding of radiolabeled bombesin (^{125}I–Tyr^4-bombesin) in primary PCa as well as in prostatic intraepithelial neoplasia. In contrast, normal and hyperplastic prostate tissue demonstrated no or very low binding of ^{125}I–Tyr^4-bombesin [8]. Consequently, various GRPr targeting radiotracers haven been investigated by several groups [9–12]. To avoid side effects provoked by agonists and because of a higher number of bindings sites, GRPr antagonists are preferred to GRPr-agonists [13]. ^{68}Ga-RM2 (also known as BAY86–7548) is an antagonistic PET tracer binding to the human GRPr on PCa cells that shows favorable kinetic and dosimetric properties and has been demonstrated to be a promising tracer for detecting of primary prostate cancer [10, 12]. However, the number of reports on GRPr imaging in recurrent PCa is still scarce [9–11, 14].

Thus, we investigated the diagnostic value of ^{68}Ga-RM2-PET/CT in patients with biochemical recurrent PCa and negative or inconclusive ^{18}FECH-PET/CT, which was the standard of care at our institution at that time. In addition, we explored the impact of variables such as PSA, antihormonal therapy (AHT) and Gleason score on diagnostic performance.

Material and methods

Design, patients

This retrospective analysis comprises 16 men with histologically confirmed PCa after primary therapy and biochemical relapse based on rising PSA. Until the end of 2013, ^{18}FECH-PET/CT was the standard of care for prostate cancer relapse at our institution. Due to the fact that we have access to RM2-PET/CT, some patients with negative or inconclusive ^{18}FECH-PET/CT were investigated with RM2-PET/CT between 2013 and 2014. When PSMA-PET/CT was newly established at our center in 2014, consequently several patients from this cohort underwent a PSMA-PET/CT.

"Negative" or "inconclusive" (i.e., only questionable findings, not deemed to justify further treatment decisions by the referring PCa specialist) results on clinical routine ^{18}FECH-PET/CT read by experienced observers led to subsequent ^{68}Ga-RM2-PET/CT on compassionate-use basis. All patients gave written informed consent for the retrospective data analysis. The retrospective data analysis was reviewed and approved by the local Ethics Committee (N° 562/15). ^{68}Ga-RM2-PET/CT was performed on a compassionate-use basis as a choline-PET/CT was negative or inconclusive. Mean age at ^{18}FECH-PET/CT and ^{68}Ga-RM2-PET/CT was 64.1 ± 8.6 and 64.6 ± 8.9 years (mean value ± standard deviation). Gleason score and PSA (ng/ml) at ^{18}FECH-PET/CT and ^{68}Ga-RM2-PET are shown in Table 1. Median time from primary PCa therapy and ^{68}Ga-RM2-PET/CT until last visit was 79.4 months and 10.9 months, respectively. Detailed information about primary therapies is shown in Supplement 1.

Synthesis of ^{68}Ga-RM2

Radiolabelling of RM2 with $^{68}GaCl_3$ was performed with the fully automated synthesis module Pharmtracer from Eckert & Ziegler (Berlin, Germany). The ^{68}Ge/^{68}Ga generator was eluted with HCl (0.1 M, 7 ml). The eluate was loaded on a cation exchange resin (Strata x-c, Phenomenex) and ^{68}Ga(III) was eluted from the cartridge into the reaction vial using a 97.6% acetone/0.02 M HCl solution. The reaction vial contained 60 μg RM2 in 2 ml sodium acetate buffer (0.2 M, pH 4.0)

Table 1 Data from ^{18}FECH-PET/CT and ^{68}Ga-RM2-PET/CT

N°	PSA [ng/ml] ^{18}F–FECH-PET/CT	AHT [Yes/No]	PSA [ng/ml] ^{68}Ga-RM2-PET/CT	AHT [Yes/No]	Time between both PET/CTs [months]	Last therapy prior to ^{68}Ga-RM2-PET/CT (except primary therapy)
1	2.4	No	4.9	No	1.7	RT prostatic fossa, hemihepatectomy (solitary lesion)
2	2.4	No	6.0	No	22.4	-
3	12.9	No	18.6	No	4.5	AHT intermittent
4	2.5	No	2.5	No	1.6	RT prostatic fossa, AHT intermittent, salvage lymph node dissection with RT
5	6.5	Yes*	6.4	Yes*	1.0	RT prostatic fossa, salvage lymph node dissection, AHT intermittent
6	26	No	226.4	No	20.3	RT prostatic fossa, AHT intermittent
7	1.8	No	7	No	13.6	RT prostatic fossa
8	1.6	Yes†	3.4	Yes†	2.4	Multiple surgeries and RTs
9	0.8	No	1.5	No	2.5	RT prostatic fossa
10	0.9	No	1.1	No	2.8	RT prostatic fossa und pelvis
11	1.6	Yes[c]	1.6	Yes‡	0.9	RT prostatic fossa, AHT intermittent
12	5	Yes‡/†	8.7	Yes‡/†	1.8	AHT, RT prostatic fossa
13	1.2	No	1.3	No	12.1	RT prostatic fossa, salvage lymph node dissection
14	7.1	No	13	No	3	-
15	4.2	Yes†	7.2	No	3.2	RT prostatic fossa, RT bone metastases, AHT
16	0.7	No	1.3	No	3.4	RT prostatic fossa, salvage radical prostatectomy, AHT

AHT = Antihormonal therapy, RT = Radiotherapy, PSA = Prostate specific antigen

*Finasteride

†Bicalutamid

‡GnRH Analoga

and 200 μl ethanol. Labeling was accomplished by heating the reaction mixture at 95 °C for 10 min. The solution was then passed over a C_{18} light cartridge (Waters, USA), washed with 3 ml saline and eluted with 1 ml 50% ethanol. The final product was constituted by addition of 7 ml saline and sterilized by filtration using Millex 0.22 μm filter (Millipore, USA). The decay-corrected yield was >90% and the radiochemical purity of the final product was >98%. The product was sterile and pyrogen-free (<0.5 EU ml^{-1}). The specific activity of ^{68}Ga-RM2 was in the range of 15–25 GBq μmol^{-1} depending on the age of the generator.

^{18}FECH-PET/CT and ^{68}Ga-RM2-PET/CT imaging

All patients fasted for at least 4 h (h) before the administration of the radiopharmaceutical and were asked to void before starting the study. For ^{18}FECH imaging patients underwent a PET scan (from base of skull to proximal thighs; 2 min. Per bed position) starting 45 min post injection (p.i.) of 255.2 ± 40.5 MBq ^{18}FECH. For ^{68}Ga-RM2 imaging patients underwent a PET scan (from base of skull to proximal thighs; 2 min per bed position) starting 1 h p.i. of 231.2 ± 63.3 MBq ^{68}Ga-RM2. Scans were either performed on a GEMINI TF PET/CT or a GEMINI TF BIG BORE PET/CT (both Philips Healthcare, Cleveland, OH). Time per bed position was 2 min. For ^{18}FECH-PET/CT and ^{68}Ga-RM2-PET/CT. PET- and CT-slice thickness was 3 mm. Depending on contraindications and availability of previous CT images, whole body CT scan was performed as contrast-enhanced diagnostic CT (120 kVp, 100–400 mAs, dose modulation) or low-dose CT (120 kVp, 25 mAs) for attenuation correction.

PET/CT-image analysis

All ^{68}Ga-RM2-PET/CTs were re-analyzed by two board-certified nuclear medicine specialists in consensus. Presence of lesions suspicious for PCa on PET images was defined as any focal uptake of ^{68}Ga-RM2 greater than physiological local background. The uptake of ^{68}Ga-RM2 was quantified by standardized uptake values (SUV) normalized to the patient's body weight. Local recurrence and metastases (LNM or other) were visually delineated in the slice with the highest tracer uptake and the SUV_{max} was recorded (in case of more than one lesion per site the lesions with the highest SUV was chosen for SUV measurement). Because of the small tumor size, no SUV_{mean} values were determined because they would have been heavily influenced by partial volume effects. For determination of background activity, a circular regions of

Table 2 Therapy and follow-up after ^{68}Ga-RM2-PET/CT

N°	Findings on ^{68}Ga-RM2-PET/CT					Confirmed **positive** RM2-scan	Non confirmed **positive** RM2-scan	**Negative** RM2-scan without positive findings during FU	**Negative** RM2-scan with positive findings during FU	Kind of confirmation / no confirmation / timepoint (*months after ^{68}Ga-RM2-PET/CT*) ***H*** = histology ***RSTD*** = response to site-directed therapy ***FI*** = further imaging	
	Findings overall positive =1 negative =0	Local relapse *(SUVmax)*	[n] Lymph node metastases *(SUVmax)*	[n] Bone metastases *(SUVmax)*	[n] Soft tissue metastases *(SUVmax)*						
1	**1**	-	**1 (hilus L)** *(5.1)*	0	**1 (lung L)** *(2.6)*	**1**				***H***	Surgical resection: pM1 (PUL, LYM 6/26, L1, V1, Pn0, R0) *(1.2 months)*
2	**1**	+ *(4.5)*	0	0	0	**1**				***RSTD***	RT of prostatic fossa (70,2 Gy), PSA-decline from 6.04 to 0.38 ng/ml (no AHT) *(2.1 months)*
3	**1**	+ *(3.5)*	0	0	0		**1**			***H / FI***	Prostate biopsy (no PCa detectable) *(0.5 months)* ^{68}Ga-HBED-PSMA-PET/CT (LNM iliaca internal L), start of AHT *(17.7 months)*
4	**1**	-	**3**† *(6.0, 7.2, 4.4)*	0	0	**1**				***H***	Salvage lymph node dissection: pN1 (10/26), PSA-decline from 2.53 to 0.67 ng/ml *(2.0 months)*
5	**1**	-	**3**‡ *(2.7, 5.5, 2.1)*	0	0	**1**				***RSTD / FI***	RT (72 Gy) of LN-region, concomitant AHT, PSA-decline from 6.44 to 0.005 ng/ml *(1.4 months)* ^{68}Ga-RM2-PET/CT (formerly suspicious LNM no longer visible) *(18.2 months)* (PSA-relapse 2.83 ng/ml)
6	**1**	+ *(5.4)*	0	0	0		**1**			-	Bone-scintigraphy (negative), start of AHT *(0.2 months)*
7	0	-	0	0	0				**1***	***FI***	Bone-scintigraphy and CT skull (isolated bone metastases skull cap) *(3.0 months)*
8	0	-	0	0	0				**1**	***FI***	^{68}Ga-HBED-PSMA-PET/CT (LNM hilus R, retropancreatic, precaval), PSA-progression to 9.3 ng/ml, restart of AHT *(4.2 months)*
9	**1**	-	**1 (iliac L)** *(2.0)*	0	0	**1**				***FI***	^{68}Ga-HBED-PSMA-PET/CT (LNM iliaca L), PSA-progression to 2.94 ng/ml, *(9.4 months)*
10	0	-	0	0	0				**1**	***FI / H***	^{68}Ga-HBED-PSMA-PET/CT (LNM iliac L and R) *(6.6 months)* Salvage lymph node dissection *(8.0 months)*
11	0	-	0	0	0	-	-	-	-	-	No further diagnostic, start of AHT with PSA stability (2.0 ng/ml)
12	**1**	-	0	**6**§ *(3.7, 2.5, 8.1, 3.0, 1.7)*	0	**1**				***FI***	^{68}Ga-HBED-PSMA-PET/CT (multiple bone metastases), PSA-progression to 8.7 ng/ml *(0.2 months)*
13	0	-	0	0	0			**1**		***FI***	^{68}Ga-HBED-PSMA-PET/CT (negative) *(10.8 months)*
14	**1**	+ *(5.1)*	0	0	0		**1**			***FI***	^{68}Ga-HBED-PSMA-PET/CT (negative) *(1.4 months)*
15	**1**	-		0	0	**1**				***FI***	

Table 2 (continued)

N°	Findings on ^{68}Ga-RM2-PET/CT					Confirmed **positive** RM2-scan	Non confirmed **positive** RM2-scan	**Negative** RM2-scan without positive findings during FU	**Negative** RM2-scan with positive findings during FU	Kind of confirmation / no confirmation / timepoint (*months after ^{68}Ga-RM2-PET/CT*)
	Findings overall positive =1 negative =0	Local relapse *(SUVmax)*	[n] Lymph node metastases *(SUVmax)*	[n] Bone metastases *(SUVmax)*	[n] Soft tissue metastases *(SUVmax)*					***H*** = histology ***RSTD*** = response to site-directed therapy ***FI*** = further imaging
			1 (obturator L) *(4.8)*							^{68}Ga-HBED-PSMA-PET/CT (LNM obturator), PSA-progression to 6.89 ng/ml, restart of AHT *(0.2 months)*
16	0	-	0	0	0		**1**		***FI***	^{68}Ga-HBED-PSMA-PET/CT (negative) *(1.6 months)*

AHT = antihormonal therapy, LNM = lymph node metastasis/metastases, RT = radiotherapy, L = left, R = right, FU = follow up, SUVmax = maximum standard uptake value

*Outside the field-of-view of the RM2-scan

†presacral L, iliac R, aortic bifurcation

‡retroperitoneal R, iliac R, presacral R

§cervical vertebral body 5, acromion L, glenoid L, chest vertebral body 6, rib 8 R, rib 10 L

interest was placed in the center of the musculus gluteus maximus (see Table 2).

Patient follow-up and reference standard

Follow-up visits and prostate cancer treatment after ^{68}Ga-RM2-PET/CT took place according to the clinical routine of the attending urologists and radiation oncologist. Clinical follow-up (last visit) for the current analysis was 10.9 ± 13.7 (median 7.0 / range: 33.4–4.6) months after ^{68}Ga-RM2-PET/CT. Median time from ^{68}Ga-RM2-PET/CT to the event of confirmation or nonconformation of ^{68}Ga-RM2-PET/CT-results (imaging, surgery, radiotherapy) was 5.3 ± 5.9 (median 2.1, range: 18.2–0-.2) months (Table 2).

Immunohistochemistry anti-GRPr/anti-BB2

Immunohistochemistry was used to confirm GRPr expression in tumor samples obtained after the ^{68}Ga-RM2-PET/CT scan. Two μm tissue slices from LNM were prepared and stained for Bombesin (BB)2 receptor. Heat-induced antigen retrieval was performed at 95 °C (30 min/pH 6.1) using Dako antigen retrieval buffer S1699. Afterwards, incubation with Rabbit anti-BB2 antibody (BB2 antibody ab39963, Lot: GR75800–1, 1:300; Abcam, UK) at room temperature (20 min) was realized followed by staining with an EnVision TM Flex Visualization system (DAKO K8000) using the AutostainerPlus (DAKO, Hamburg, Germany). After washing (PBS and water), counterstaining with hematoxylin and eosin was performed. Staining of normal pancreas parenchyma was used as a positive control for BB2 receptors.

Statistics

Descriptive statistics was done by calculating mean ± standard deviation (SD), median and interquartile range (IQR). Due to the lack of Gaussian distribution (D'Agostino & Pearson omnibus normality test) continuous variables were compared with a two-sided Mann-Whitney test or with a two-sided Wilcoxon matched-pairs signed rank test. Categorical variables were compared by Fischer's exact test. All statistics were done with GraphPad Prism 6.

Results

Patient characteristics / history

Mean initial PSA at diagnosis of PCa was 18.5 ± 13.5 ng/ml (median 14.4 ng/ml). Seven of 16 men had a T2 stage (43.8%) and 9/16 a T3 stage (56.2%) (Supplement 1). Radical

prostatectomy including lymphadenectomy or radiotherapy as primary therapy were performed in 13/16 (81.3%) and 3/16 men (18.7%), respectively (Supplement 1). After 21.2 ± 36.0 months (median 9.5 months, IQR 19.3) the first biochemical recurrence was observed in all 16 men. Thereupon in 14/16 men at least one salvage therapy was conducted (Table 1). The 13/14 men had a radiotherapy of the prostatic fossa, (6/14) had a metastases-directed therapy. Intermittent AHT was administered in 8/16 men prior to ^{68}Ga-RM2-PET/CT.

Clinical setting at ^{18}FECH-PET/CT and ^{68}Ga-RM2-PET/CT

^{18}FECH-PET/CT was performed in all patients to localize recurrent PCa in the setting of biochemical recurrence. Fourteen of 16 ^{18}FECH-PET/CTs did not show any pathologic findings, 2/16 were inconclusive (Table 2). ^{68}Ga-RM2-PET/CT was performed mean 6.1 ± 6.8 months (median 2.9 months, IQR 8.5) after ^{18}FECH-PET/CT. Mean 4.7 ± 3.0 (median 4.1) years after the event of first biochemical relapse the ^{68}Ga-RM2-PET/CT was conducted.

Median PSA at time of ^{18}FECH-PET/CT (2.4 ng/ml, IQR 4.9 ng/ml, range: 0.74–26 ng/ml) was significantly lower as compared to median PSA at ^{68}Ga-RM2-PET/CT (5.5 ng/ml, IQR 6.8, range: 1.06–226.4 ng/ml). AHT was administered to 4/16 (25%) of the patients at time of ^{68}Ga-RM2-PET/CT and to 5/16 (31.3%) at the time of ^{18}FECH-PET/CT (p = 1.00).

Findings on ^{68}Ga-RM2-PET/CT

After injection of RM2, no side effects were observed. Results of all 16 ^{68}Ga-RM2-PET/CTs are summarized in Table 2. Overall, ^{68}Ga-RM2-PET/CT was abnormal in ten of the 16 patients (63%). The two patients with inconclusive results on ^{18}FECH-PET/CT (patients N°4, N°12) showed clearly positive findings on ^{68}Ga-RM2-PET/CT: The ^{18}FECH-PET/CT of patient N°4 depicted only mild tracer accumulation in left presacral lymph nodes (LN) and LNs at the aortic bifurcation, whereas the ^{68}Ga-RM2-PET/CT revealed intense tracer accumulation in these LNs (presacral/aortic bifurcation) and additional focal tracer uptake of right iliac LNs. In patient N°12, the ^{68}Ga-RM2-PET/CT showed multiple bone lesions (e.g., scapula, sternum, vertebral body, acromion) while there was only one mildly metabolically active lesion on ^{18}FECH-PET/CT. SUVs (standard uptake values) of lesions suspicions for local recurrence or metastases ranged from 2.0 to 8.1 (Table 2). Mean SUV of background (musculus gluteus maximus) was 0.38 ± 0.09.

Impact of PSA-level, Gleason score and antihormonal therapy on ^{68}Ga-RM2-PET/CT

Patients with a positive ^{68}Ga-RM2 scan showed a significantly higher PSA value (median 6.8 ng/ml, IQR 10.2 ng/ml, range: 1.53–226.4 ng/ml) than those with a negative scan (median 1.5 ng/ml, IQR 3.1 ng/ml, range: 1.06–7.0 ng/ml (p = 0.016) (Fig. 1a). Distribution of Gleason scores (stratified as low-risk vs. high-risk cases, i.e., Gleason 6-7a vs. Gleason 7b-9) showed no significant association (p = 0.63) with negative and positive results on ^{68}Ga-RM2-PET/CT (Fig. 1b). The 2/10 of the patients with a positive ^{68}Ga-RM2-PET/CT were under AHT compared to 2/6 of the men with a negative ^{68}Ga-RM2-PET/CT (p = 0.60) (Table 1).

Confirmation of ^{68}Ga-RM2-PET/CT results

For the ten men with a positive ^{68}Ga-RM2-PET/CT, the findings were confirmed by surgical resection and histology of the lesions (n = 2; including immunohistochemistry, see Fig. 3A, B), by response to site-directed therapies (n = 2) or by further imaging (n = 3) (Table 2). For the remaining three men with a positive ^{68}Ga-RM2-PET/CT, the presence of PCa manifestations was not confirmed in two patients (biopsy with further imaging n = 1, further imaging n = 1) and is still unclear in one patient (mean follow-up 10.9 ± 13.7 (median 7.0) months) (Table 2). All these non-confirmed positive findings concerned patients with suspected local recurrence (i.e., three of four cases with suspected local recurrence in total) (Table 2).

^{68}Ga-RM2-PET/CT was negative in six patients. A subsequent ^{68}Ga-HBED-CC-PSMA-PET/CT was also negative in two of these patients. In two additional cases, a ^{68}Ga-HBED-CC-PSMA-PET/CT strongly suggested the presence of LNM. Of the remaining two patients with a negative ^{68}Ga-RM2-PET, one patient showed the unusual finding of a

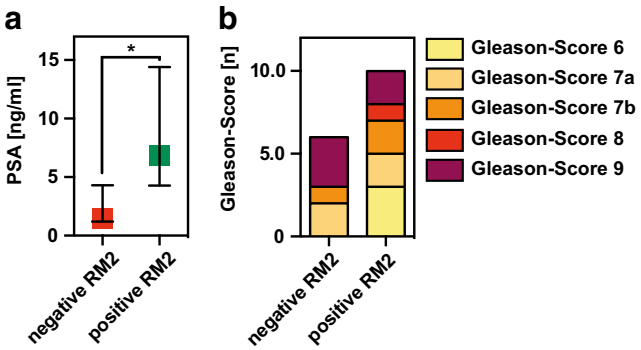

Fig. 1 (**a**) PSA values (median 1.5 ng/ml) in patients with negative results of ^{68}Ga-RM2-PET/CT was significantly lower than PSA values (median 6.8 ng/ml) of patients with positive results (p = 0.016). (**b**) Distribution of Gleason scores showed no association with negative and positive findings on ^{68}Ga-RM2-PET/CTs (p = 0.63; stratified as low-risk vs. high-risk cases, i.e. Gl. 6-7a vs. Gl. 7b-9) (p = 0.63)

solitary bone metastasis of the skull on bone-scan and CT, while the skull has been outside the field of view (FOV) in the ^{68}Ga-RM2- and the ^{18}FECH-PET/CT. The remaining patient with negative ^{68}Ga-RM2-scan received no further imaging studies but received AHT and showed a stable PSA at the level of 2 ng/ml (Table 2).

As representative examples, ^{68}Ga-RM2- and ^{18}FECH-PET and corresponding CT sections of patients N° 4, 5 and 12 are shown in Fig. 2a-c. Because of detected presacral and iliac LNM by ^{68}Ga-RM2-PET/CT, patient N°4 underwent a salvage lymph node dissection with histological confirmation of these lesions. He had a PSA decline after therapy (Fig. 2a). In patient N°5 (Fig. 2b) a salvage radiotherapy of suspicious iliac and presacral LNM was performed which was followed by a marked PSA decline (Fig. 3).

Discussion

The aim of our investigation was to explore the value of GRPr-PET in a selected population of patients with biochemically recurrent PCa with prior non-contributory (negative/inconclusive) ^{18}FECH-PET/CT using the ^{68}Ga-labeled GRPr antagonist RM2. We demonstrate that even in this highly selected, most challenging cohort of patients, ^{68}Ga-RM2-PET/CT detected at least one region with focal pathological uptake in 62.5% of the patients (10/16). In seven of these ten cases, positive findings were confirmed by histopathology or by clinical follow-up (Table 2).

Literature on PET-Imaging using GRPr ligands in patients with PCa-recurrence and information about true-positive (confirmed) lesions are rare and largely different (also due to the different tracers used). In the literature, the number of individuals with PCa relapse undergoing a RM2-PET/CT is apparently low (Kähkönen et al. n = 3 [10], Minamimoto et al. n = 7 [14], Mather et al. n = 6 [15], Sah et al. n = 5 [11]). So far, data from 21 men with PCa recurrence undergoing PET imaging with various radiolabeled GRPr ligands (among others ^{68}Ga-RM2) are available [10, 11, 14, 16], and we were able to contribute the clinical data from 16 PCa patients undergoing a ^{68}Ga-RM2-PET/CT. Considering these 21 patients and the patients from our cohort (n = 16), pathological focal uptake on GRPr-PET was detected in 23/37 cases. The majority of the patients (11/23 = 48%) had the suspicion of lymph node metastases (LNM) alone, while only 2/23 (9%) and 4/23 (17%) patients where suspected to suffer from both LNM and bone metastases (BM), and BM alone, respectively (among other manifestations and combinations). Information on a confirmation of these lesions is not completely given in the published studies [10, 11, 14, 16]. Furthermore, the available data from the 14 patients (literature and our cohort) without suspicious findings on GRPr-PET indicates that in 6/14 (43%) patients bone metastases were finally discovered by

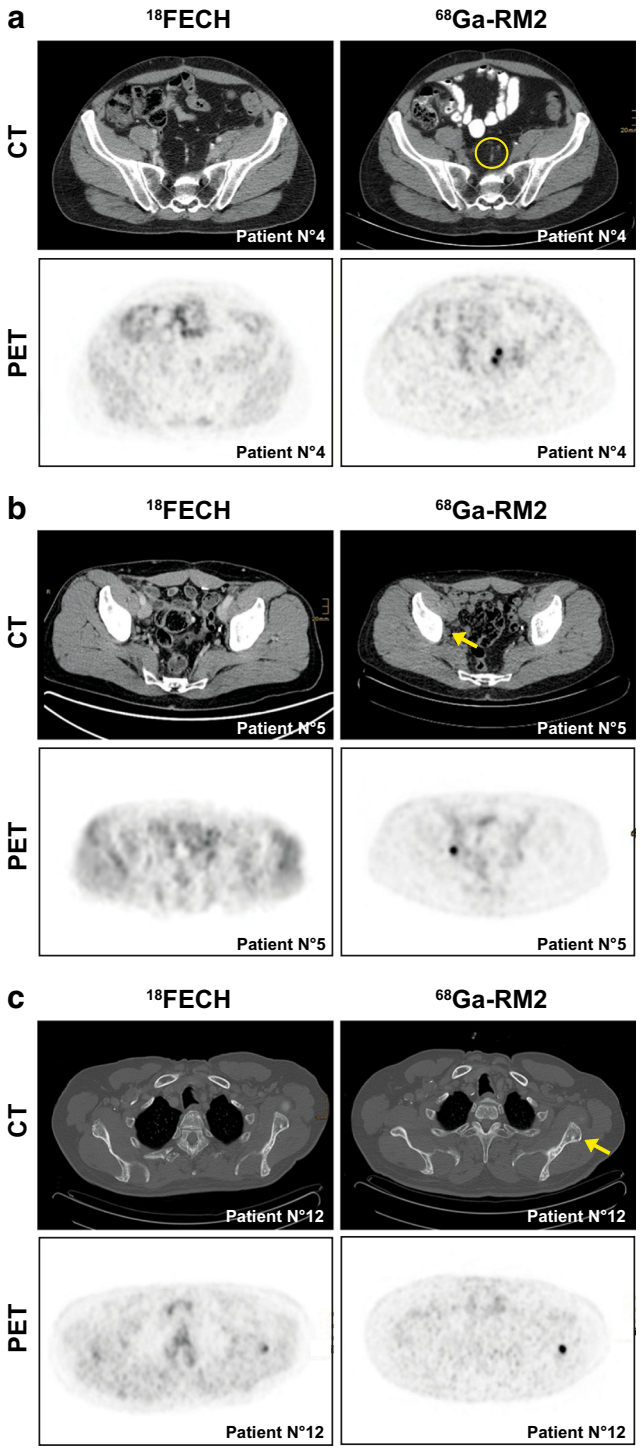

Fig. 2 ^{18}FECH-PET/CT and ^{68}Ga-RM2-PET with corresponding CT from three patients. (**a**) In patient N°4 ^{68}Ga-RM2-PET/CT uncovered presacral and parailiac (not shown) LNM. (**b**) In patient N°5 ^{68}Ga-RM2-PET/CT detected parailiac and presacral (not shown) LNM. (**c**) In patient N°12 ^{68}Ga-RM2-PET/CT showed multiple bone lesions while ^{18}FECH-PET/CT showed only one lesion with slight tracer uptake in the left scapula

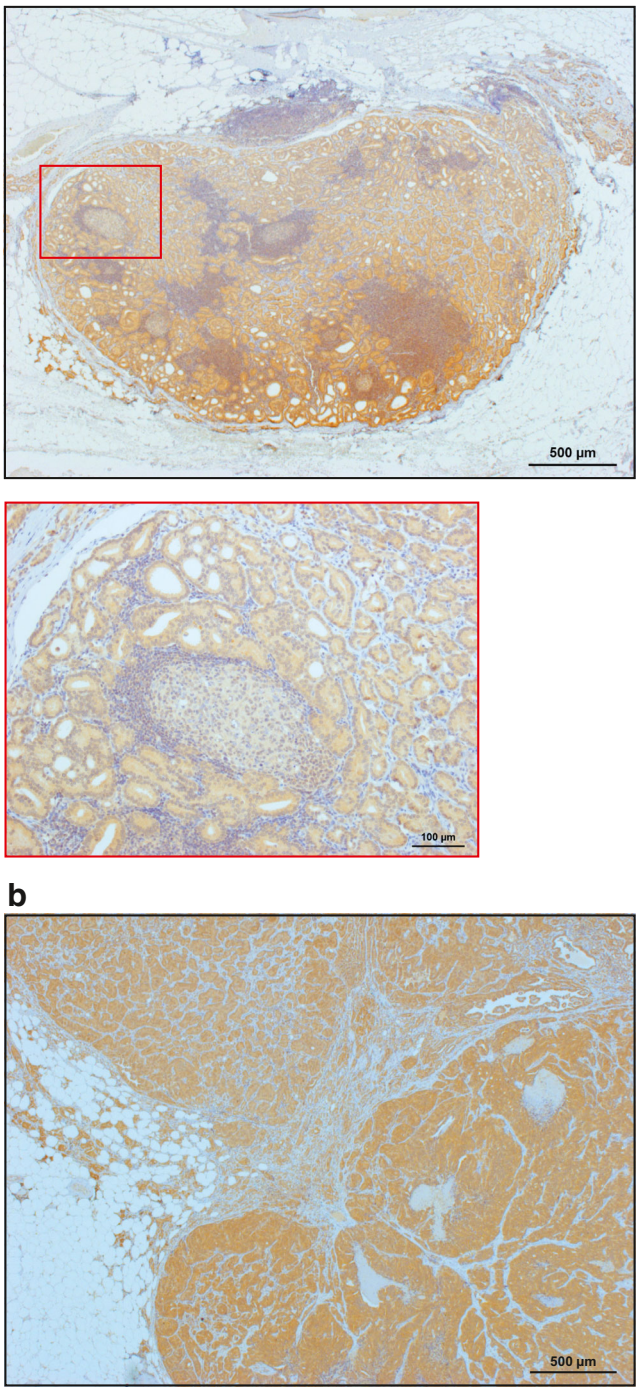

Fig. 3 Positive immunohistochemistry for GRP-receptor (BB2-receptor). (**a**) Resected lung hilus LNM (patient N°1). (**b**) Resected pelvic LNM (patient N°4)

follow-up imaging (for comparison: 3/14 (21.5%) local relapses, 2/14 (14%) LNM, 3/14 (21.5%) no suspicious lesion) [10, 11, 14, 16]. In our cohort only 1/16 individuals showed bone metastases in the PET FOV; in this patient ^{68}Ga-RM2-PET detected more bone lesions than ^{18}FECH-PET/CT (Table 1). Taken together, these findings suggest that GRPr PET may be of limited sensitivity for the detection of bone metastases in PCa which warrants further investigation.

We note that most of the true positive GRPr PET/CT findings were observed in lymph node metastases, but this may be due to patient selection. Only two patients developed osseous metastases during follow-up. In one patient these metastases were detected by GRPr PET/CT; in the other patient a solitary skull metastasis was outside of the field-of-view of the GRPr PET/CT scan. Two presumable false positive findings were observed in the prostate bed, these may be caused by artifacts from activity in the urinary bladder.

Generally, imaging in cases of biochemical PCa-recurrence with PSA-values below 1 ng/ml and a slow PSA-doubling time (suspicion of local relapse in the prostate fossa) is of course a relevant clinical issue in order to verify or exclude local relapse of PCa-recurrence. Furthermore, imaging (e.g., with RM2-PET/CT or PSMA-PET/CT) at PSA-levels clearly >1 ng/ml (e.g., median PSA 5.45 ng/ml in our cohort) is of great importance in order to follow the strategy of metastasis-directed therapy such as salvage lymph node dissection or target radiotherapy increasingly performed in many European countries [17].

It is difficult to state a clear association between the PSA level and the probability of positive findings of ^{68}Ga-RM2-PET/CT. In our study we observed that the median PSA level was significantly higher in patients with positive findings compared to those with negative findings on ^{68}Ga-RM2-PET/CT. Pooling the available data from the literature and our study, PSA values of patients with positive GRPr PET scans (n = 23) ranged from 0.36–226.4 ng/ml (mean: 22 ng/ml, median: 7.2 ng/ml), PSA from negative GRPr PET scans (n = 14) ranged from 1.06–282 ng/ml (mean: 56.7 ng/ml, median 6.9 ng/ml) [10, 11, 14, 16], thus showing a wide overlap.

Data about comparison of PET-CT results from ^{68}Ga-RM2-PET/CT versus ^{68}Ga-HBED-PSMA-PET/CT are scarce. Four of six patients with negative ^{68}Ga-RM2-PET/CT also underwent ^{68}Ga-HBED-PSMA-PET/CT during follow-up (mean 5.8 ± 3.9, median 5.4 months after ^{68}Ga-RM2-PET/CT), which was also negative in two patients, while in two patients ^{68}Ga-HBED-PSMA-PET/CT suggested the presence of LNM. In contrast to this sequential imaging approach, Minamimoto et al. investigated seven patients with recurrent PCa using both ^{68}Ga-RM2-PET/CT and ^{68}Ga-HBED-PSMA-PET/CT within mean 42.9 ± 25.2 days (range 13–85 days) [14]. They found that the locations of suspected metastases were almost the same for both tracers (n = 5 LNM, n = 1 LNM + bone metastases, n = 1 local relapse detected only by ^{68}Ga-HBED-PSMA- but not ^{68}Ga-RM2-PET/CT). Periaortal LNM were more clearly visualized by ^{68}Ga-RM2-PET/CT in two patients due to the lack of tracer accumulation in the small intestine. These findings underline that additional work, such as

imaging studies using both tracers, is needed to understand the expression of PSMA and GRPr in different types and stages of prostate cancer and the merits of the respective molecular imaging techniques [14].

Limitations

Limitations of our study are the small number of patients investigated, the heterogeneity of the patients concerning PCa stages, tumor burden, PSA level and Gleason score at time of biochemical relapse and that most of the findings were not histologically confirmed. Due to the nature of PCa, even if recurrent, most patients present with considerable survival rates associated with several differential PCa therapies in their history (different adjuvant therapies after primary therapy and prior to RM2-PET/CT), this also caused heterogeneity in our cohort.

Furthermore, the time between ^{18}FECH-PET and ^{68}Ga-RM2-PET/CT (mean 6.1 ± 6.8 months) and the higher PSA level at ^{68}Ga-RM2-PE[18] T/CT might cause bias in results. One cannot exclude that at least some of the initial ^{18}FECH-PET-negative patients would have been positive in a follow-up ^{18}FECH-PET scan after this time period. Performance of ^{68}Ga-RM2-PET/CT only in those patients with preceding negative/inconclusive ^{18}FECH-PET/CT represents a selection of highly challenging patients which probably reduced the chance of positive findings on the subsequent ^{68}Ga-RM2-PET/CT. This suggests that the true detection rate might have been higher in a more general population of PCa patients with biochemical relapse.

Conclusion

In the scenario of PCa relapse and a negative/inconclusive ^{18}FECH-PET/CT, a consecutive ^{68}Ga-RM2-PET/CT generated important information for clinical decision-making in the majority of the patients examined in our study. Thus, ^{68}Ga-RM2 is potentially a valuable new PET tracer for imaging PCa relapse. Larger prospective clinical trials are warranted to determine the role of ^{68}Ga-RM2-PET/CT for PCa imaging in the clinical setting.

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Compliance with ethical standards All procedures performed in studies involving human participants 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 study was reviewed and approved by the local Ethics Committee (N° 562/15). Informed consent was obtained from all individual participants included in the study.

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References

1. Han M, Partin AW, Zahurak M. Biochemical (prostate specific antigen) recurrence probability following radical prostatectomy for clinically localized prostate cancer. J Urol. 2003;169(2):517–23. doi:10.1097/01.ju.0000045749.90353.c7.
2. Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol. 2014;65(1):124–37. doi:10.1016/j.eururo.2013.09.046.
3. Hovels AM, Heesakkers RA, Adang EM. The diagnostic accuracy of CT and MRI in the staging of pelvic lymph nodes in patients with prostate cancer: a meta-analysis. Clin Radiol. 2008;63(4):387–95. doi:10.1016/j.crad.2007.05.022.
4. Picchio M, Briganti A, Fanti S, Heidenreich A, Krause BJ, Messa C, et al. The role of choline positron emission tomography/computed tomography in the management of patients with prostate-specific antigen progression after radical treatment of prostate cancer. Eur Urol. 2011;59(1):51–60. doi:10.1016/j.eururo.2010.09.004.
5. Cimitan M, Evangelista L, Hodolic M, Mariani G, Baseric T, Bodanza V, et al. Gleason score at diagnosis predicts the rate of detection of 18F-choline PET/CT performed when biochemical evidence indicates recurrence of prostate cancer: experience with 1,000 patients. J Nucl Med. 2015;56(2):209–15. doi:10.2967/jnumed.114.141887.
6. Jadvar H. Molecular imaging of prostate cancer: PET radiotracers. AJR Am J Roentgenol. 2012;199(2):278–91. doi:10.2214/AJR.12.8816.
7. Jensen RT, Battey JF, Spindel ER, Benya RV. International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. Pharmacol Rev. 2008;60(1):1–42. doi:10.1124/pr.107.07108.
8. Markwalder R, Reubi JC. Gastrin-releasing peptide receptors in the human prostate: relation to neoplastic transformation. Cancer Res. 1999;59(5):1152–9.
9. Wieser G, Mansi R, Grosu AL, Schultze-Seemann W, Dumont-Walter RA, Meyer PT, et al. Positron emission tomography (PET) imaging of prostate cancer with a gastrin releasing peptide receptor antagonist–from mice to men. Theranostics. 2014;4(4):412–9. doi:10.7150/thno.7324.
10. Kahkonen E, Jambor I, Kemppainen J, Lehtio K, Gronroos TJ, Kuisma A, et al. In vivo imaging of prostate cancer using [68Ga]-labeled bombesin analog BAY86-7548. Clin Cancer Res. 2013;19(19):5434–43. doi:10.1158/1078-0432.CCR-12-3490.
11. Sah BR, Burger IA, Schibli R, Friebe M, Dinkelborg L, Graham K, et al. Dosimetry and first clinical evaluation of the new 18F-radiolabeled bombesin analogue BAY 864367 in patients with prostate cancer. J Nucl Med. 2015;56(3):372–8. doi:10.2967/jnumed.114.147116.
12. Roivainen A, Kahkonen E, Luoto P, Borkowski S, Hofmann B, Jambor I, et al. Plasma pharmacokinetics, whole-body distribution, metabolism, and radiation dosimetry of 68Ga bombesin antagonist BAY 86-7548 in healthy men. J Nucl Med. 2013;54(6):867–72. doi:10.2967/jnumed.112.114082.

13. Mansi R, Wang X, Forrer F, Waser B, Cescato R, Graham K, et al. Development of a potent DOTA-conjugated bombesin antagonist for targeting GRPr-positive tumours. Eur J Nucl Med Mol Imaging. 2011;38(1):97–107. doi:10.1007/s00259-010-1596-9.
14. Minamimoto R, Hancock S, Schneider B, Chin F, Jamali M, Loening AM, et al. Pilot comparison of 68Ga-RM2 PET and 68Ga-PSMA PET in patients with biochemically recurrent prostate cancer. J Nucl Med. 2015; doi:10.2967/jnumed.115.168393.
15. Mather SJ, Nock BA, Maina T, Gibson V, Ellison D, Murray I, et al. GRP receptor imaging of prostate cancer using [Tc]Demobesin 4: a first-in-man study. Mol Imaging Biol. 2014; doi:10.1007/s11307-014-0754-z.
16. Mather SJ, Nock BA, Maina T, Gibson V, Ellison D, Murray I, et al. GRP receptor imaging of prostate cancer using [(99m)Tc]Demobesin 4: a first-in-man study. Mol Imaging Biol. 2014;16(6):888–95. doi:10.1007/s11307-014-0754-z.
17. Pfister D, Porres D, Heidenreich A, Heidegger I, Knuechel R, Steib F, et al. Detection of recurrent prostate cancer lesions before salvage lymphadenectomy is more accurate with (68)Ga-PSMA-HBED-CC than with (18)F-Fluoroethylcholine PET/CT. Eur J Nucl Med Mol Imaging. 2016;43(8):1410–7. doi:10.1007/s00259-016-3366-9.
18. Rigatti P, Suardi N, Briganti A. Pelvic/retroperitoneal salvage lymph node dissection for patients treated with radical prostatectomy with biochemical recurrence and nodal recurrence detected by [11C]choline positron emission tomography/computed tomography. Eur Urol. 2011;60(5):935–43. doi:10.1016/j.eururo.2011.07.060.