^{68}Ga-PSMA-PET/CT in Patients With Biochemical Prostate Cancer Recurrence and Negative ^{18}F-Choline-PET/CT

Christina Bluemel, MD, Markus Krebs, MD,† Bülent Polat, MD,‡ Fränze Linke, MD,§ Matthias Eiber, MD,|| Samuel Samnick, PhD,* Constantin Lapa, MD,* Michael Lassmann, PhD,* Hubertus Riedmiller, MD,† Johannes Czernin, MD,¶ Domenico Rubello, MD,# Thorsten Bley, MD,** Saskia Kropf,†† Hans-Juergen Wester, PhD,‡‡ Andreas K. Buck, MD,* and Ken Herrmann, MD*¶*

Approximately one third of prostate cancer patients experience biochemical recurrence within 5 years of primary curative treatment.[1] A variety of treatment options including salvage radiation therapy and/or systemic treatment are available for these patients.[2] Salvage radiotherapy after biochemical recurrence after radical prostatectomy improved biochemical recurrence-free survival in patients with serum PSA levels greater than 0.5 ng/mL.[3] Moreover, prostate cancer specific survival improved after salvage radiotherapy if the PSA doubling time is less than 6 months.[4] Therefore, localization of recurrent disease is important as it may enable early and/or personalized rational therapeutic interventions that may lead to improved outcomes.

Several imaging modalities including computed tomography (CT), magnetic resonance imaging (MRI) and transrectal ultrasound (TRUS) are used to identify sites of recurrence.[5] PET imaging using probes of lipid and amino acid metabolism has also been used successfully to detect recurrent disease and has shown impact on patient management.[5,6] However, when serum PSA levels are less than 3 ng/mL, recurrence sites are only detected in 40% to 60% of patients.[7–10]

Expression of the transmembrane folate hydrolase prostate-specific membrane antigen (PSMA) has been described in normal and hyperplastic prostate tissue, prostatic intraepithelial neoplasia, and invasive carcinomas.[11–13] Moreover, elevated PSMA expression in prostate cancer carries a poor prognosis.[14] Different groups have reported the successful radiolabeling of peptide ligands that specifically bind to PSMA.[15,16] ^{68}Ga-PSMA-PET/CT detects sites of recurrence with high accuracies ranging from 74% to 89%.[17–19] However, ^{68}Ga-labelled compounds are produced in generators that provide limited activity per synthesis. Thus, depending on the age of the generator, only 1 to 4 patient doses per elution can be produced. In contrast, more than 10 times the activity of ^{18}F-choline can be provided by one cyclotron production.

To optimize resource utilization, we developed a sequential clinical imaging approach as follows: patients with biochemical relapse after primary curative treatment underwent an ^{18}F-choline-PET/CT scan first. If this scan was negative, patients were offered an additional ^{68}Ga-PSMA-PET/CT using EuK-Sub-kff-^{68}Ga-DOTAGA.[20] The aim of this sequential molecular imaging approach was to determine whether the addition of

From the Departments of *Nuclear Medicine, †Urology, and ‡Radiation Oncology, University Hospital of Würzburg, Würzburg; §Department of Radiation Oncology, Klinikum Ansbach, Ansbach; ||Department of Nuclear Medicine, Technische Universität München, Munich, Germany; ¶Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA; #Department of Nuclear Medicine-PET/CT Oncologic & Endocrine Sections, Rovigo Hospital, Rovigo, Italy; **Department of Radiology, University Hospital of Würzburg, Würzburg, Germany; ††Scintomics GmbH, Fürstenfeldbruck; and ‡‡Pharmaceutical Radiochemistry, Technische Universität München, Munich, Germany

Conflicts of interest and sources of funding: S.K. and H.J.W. are CEOs and shareholders of Scintomics, the company distributing PSMA-directed peptide ligands. Both coauthors have not influenced data acquisition or analysis but contributed to conception/design, drafting of manuscript, administrative, technical or material support, and supervision.

Author contributions: Conception and design: C.B., K.H., A.K.B., H.J.W., and J.C. Acquisition of data: C.B., C.L., M.L., and K.H. Analysis and interpretation of data: C.B., B.P., M.K., F.L., C.L., T.B., and K.H. Drafting of manuscript: C.B., J.C., C.L., A.K.B., H.J.W., and K.H. Critical revision of manuscript: M.E., D.R., J.C., and K.H. Administrative, technical, or material support: M.W., S.K., S.S., and H.J.W. Supervision: S.S., M.L., H.R., D.R., A.K.B., and H.J.W.

Correspondence to: Ken Herrmann, MD, University Hospital Würzburg, Department of Nuclear Medicine Oberdürrbacher Str. 6, Würzburg, Germany. E-mail: herrmann_k1@ukw.de.

TABLE 1. Site of Recurrence (n = 125 Patients)*

Choline-Positive Lesions (n = 93 Patients)	n (%)
LR	19 (20%)
LN	42 (45%)
Bone	13 (14%)
LR and LN	4 (4%)
LR and bone	2 (2%)
LN and bone	7 (8%)
LR, LN, and bone	2 (2%)
Distant metastases	4 (4%)
PSMA-positive lesions (n = 14/32 patients)	
LR	8 (57%)
LN	6 (43%)

*Patients, who refused additional ^{68}Ga-PSMA-PET/CT (n = 9) and patients with equivocal findings (n = 5) were excluded. LR indicates local recurrence; LN, lymph node metastases; bone, bone metastases.

a ^{68}Ga-PSMA-PET/CT scan in patients with biochemical recurrence and a negative ^{18}F-choline scan provided incremental diagnostic value.

MATERIALS AND METHODS

Sequential Molecular Imaging Approach

The sequential imaging approach is used routinely in our clinic commences with an ^{18}F-choline-PET/CT. In case of a positive choline-result, no further imaging is performed. Patients with a negative ^{18}F-choline study are offered an additional ^{68}Ga-PSMA-PET/CT study. Informed consent for the clinical scans was obtained from all individuals. Because of the retrospective design, a need for formal review was waived by the ethics committee of the Universitätsklinikum Würzburg, Germany.

Patients

From January 2014 to May 2015, 139 consecutive patients with biochemical relapse defined as serum PSA values of 0.2 ng/mL or greater after radical prostatectomy or 2 ng/mL or greater in patients treated with radiation therapy were offered the sequential imaging approach. The mean patient age was 69.4 ± 6.8 years (range, 46.8–83.0 years). Initial treatment included radical prostatectomy (RPT; 58 patients; 42%), radiotherapy (24 patients; 17%), or a combination of both (56 patients; 40%). One patient (1%) underwent focused high intensity ultrasound ablation (HIFU). The mean serum PSA level at recurrence was 5.4 ± 12.73 ng/mL (range, 0.20–126.56 ng/mL; median, 1.96 ng/mL). The PSA doubling time was 9.9 ± 10.6 months, and the PSA velocity was 7 ± 25 ng/mL per year.

Choline-PET/CT

^{18}F-choline was synthesized as previously described.[21] Images were acquired using a Biograph mCT 64 (Siemens Medical Solutions, Germany). Patients fasted for at least 4 hours before the PET/CT scans. 311 ± 27 MBq ^{18}F-choline (range, 229–385 MBq) were injected intravenously, and patients received 10 mg of furosemide at the same time. Sixty minutes later, the emission data were acquired from the base of skull, or the vertex, to the proximal thighs (2–3 minutes per bed position). Subsequently, contrast-enhanced CT (CECT) images were acquired. After decay and scatter correction, PET data were reconstructed iteratively with attenuation correction using dedicated software (HD·PET; Siemens e-soft, Germany).

PSMA-PET/CT

^{68}Ga-PSMA I&T was synthesized as described previously.[16] A mean of 133 ± 20 MBq (range, 79–161 MBq) of ^{68}Ga-PSMA was injected. This was followed 60 minutes later by a low-dose CT acquisition for attenuation correction and lesion localization. PET emission data were then acquired for 2 to 3 minutes per bed position. PET data were also corrected for dead-time, random events, and scatter.

Image Analysis

PET/CT images were visually analyzed by 3 experienced nuclear physicians (C.B., C.L., and K.H.) for the presence and localization of suspicious lesions; in doubtful cases, diagnosis was reached by consensus. In PET, any focal uptake higher than the surrounding background and not associated with physiological uptake

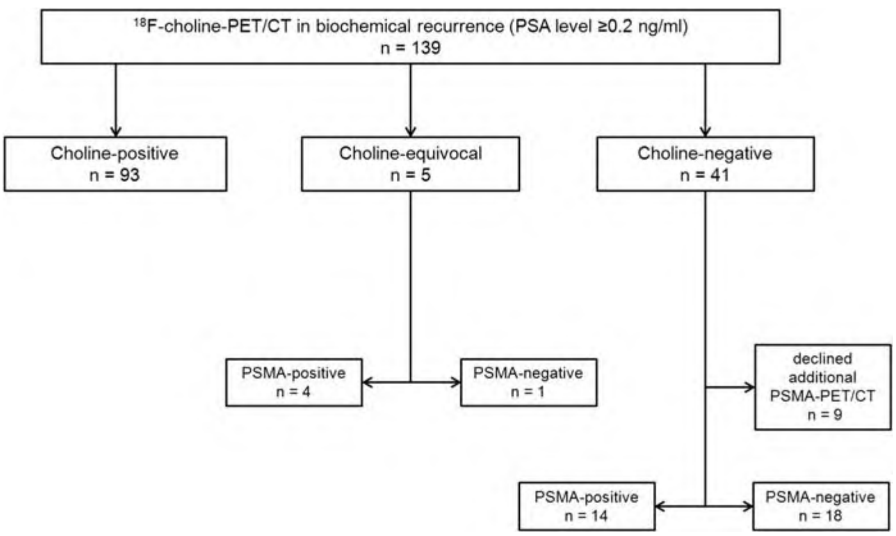

FIGURE 1. Flow diagram: sequential imaging approach (n = 139 patients).

was considered suspicious. Lesions were classified as malignant, equivocal, or benign. Visual findings were rated as equivocal if the uptake was not typical for metastasis or local recurrence but nevertheless unclear. Lesions were further classified by localization as listed in Table 1.

Statistical Analysis

Descriptive analysis was performed by calculating the median, mean, standard deviation, and range. PSA kinetics were calculated according to Pound et al.[22] The detection rate on a per patient basis (patients with at least 1 positive finding) was plotted against the absolute PSA value and PSA kinetics according to previously published studies.[7,19] Two-sample *t* test was used to evaluate differences in the Gleason score between subgroups. The Mann-Whitney *U* test was used to evaluate differences in PSA kinetics among subgroups with and without pathological uptakes. All tests were performed 2-sided, and a level of significance of $\alpha = 5\%$ was used. Statistical analyses were conducted with SPSS statistics software (version 22.0; SPSS, Inc., Chicago, IL).

RESULTS

Sequential imaging findings: Data of 139 consecutive patients were analyzed. The clinical report was positive in 93 (66.9%), negative in 41 (29.5%), and equivocal in 5 (3.6%) patients. Approximately 32 (78.0%) of 41 patients with a negative ^{18}F-choline-PET/CT agreed to undergo an additional ^{68}Ga-PSMA-scan, whereas 9 (22.0%) of 41 declined participation (Fig. 1). The time interval between ^{18}F-choline- and ^{68}Ga-PSMA-PET/CT was 19 ± 16 days (range, 5–51 days; median, 11 days).

Patients with an equivocal ^{18}F-choline study (n = 5) were excluded from the overall analysis. However, because all 5 patients with equivocal ^{18}F-choline-PET/CT scans underwent an additional ^{68}Ga-PSMA scan, their findings are mentioned separately (see below in "Results" section). Those patients who declined the ^{68}Ga-PSMA PET/CT study (n = 9) were excluded from further analysis. Thus, data from 125 patients were analyzed.

Detection rates and lesion localization: The overall detection rate of the sequential molecular imaging approach was 85.6% (107/125). ^{18}F-choline-PET/CT detected disease recurrence in 74.4% (93/125), whereas additional ^{68}Ga-PSMA-PET/CT identified recurrence in 14 of 32 patients with negative choline scans (43.8%). Thus, the overall detection rate increased by 11.2% (14/125). For details, see Figure 2 and Table 2. Choline-negative but ^{68}Ga-PSMA-positive lesions occurred in the prostatic bed in 8(25.0%) and in lymph nodes in 6 (18.8%) of the 32 patients (Table 1).

Serum PSA level: The sequential imaging approach and ^{18}F-choline-PET/CT alone detected recurrence best when PSA levels were 2 ng/mL or greater. Corresponding detection rates were 97.0% (64/66) for the sequential imaging approach and 89.4% (59/66) for ^{18}F-choline alone.

Detection rates decreased with lower PSA values. For the sequential imaging approach, they were 81.8% (27/33) for PSA values of 1 to 2 ng/mL and 61.5% (16/26) for PSA values of 0.2 ng/mL or greater to less than 1 ng/mL. Detection rates for ^{18}F-choline-PET/CT were lower at 66.7% for PSA of 1 to 2 ng/mL and 46.1% for PSA levels of 0.2 ng/mL or greater to less than 1 ng/mL. More detailed results are displayed in Figure 2 and Table 2.

The highest rate of positive ^{68}Ga-PSMA findings occurred when PSA was greater than 2 ng/mL (Figs. 3 and 4). Approximately 71.4% (5/7) of these patients had positive ^{68}Ga-PSMA scans. When PSA levels were 1 to 2 ng/mL and 0.2 ng/mL or greater to less than 1 ng/mL, ^{68}Ga-PSMA detection rates were 45.5% (5/11) and 28.6% (4/14), respectively.

PSA doubling time and PSA velocity: Detection rates of sequential imaging were unrelated to PSA doubling time. The highest detection rate was found in patients with PSA doubling time of greater than 4 to 6 months (95.7%; 22/23).

In patients with PSA velocity of 5 ng/mL per year or greater, ^{18}F-choline-PET/CT successfully detected sites of recurrence in all patients (n = 27). The added value of ^{68}Ga-PSMA-PET/CT was highest in patients with a PSA velocity of 2 to less than 5 ng/mL

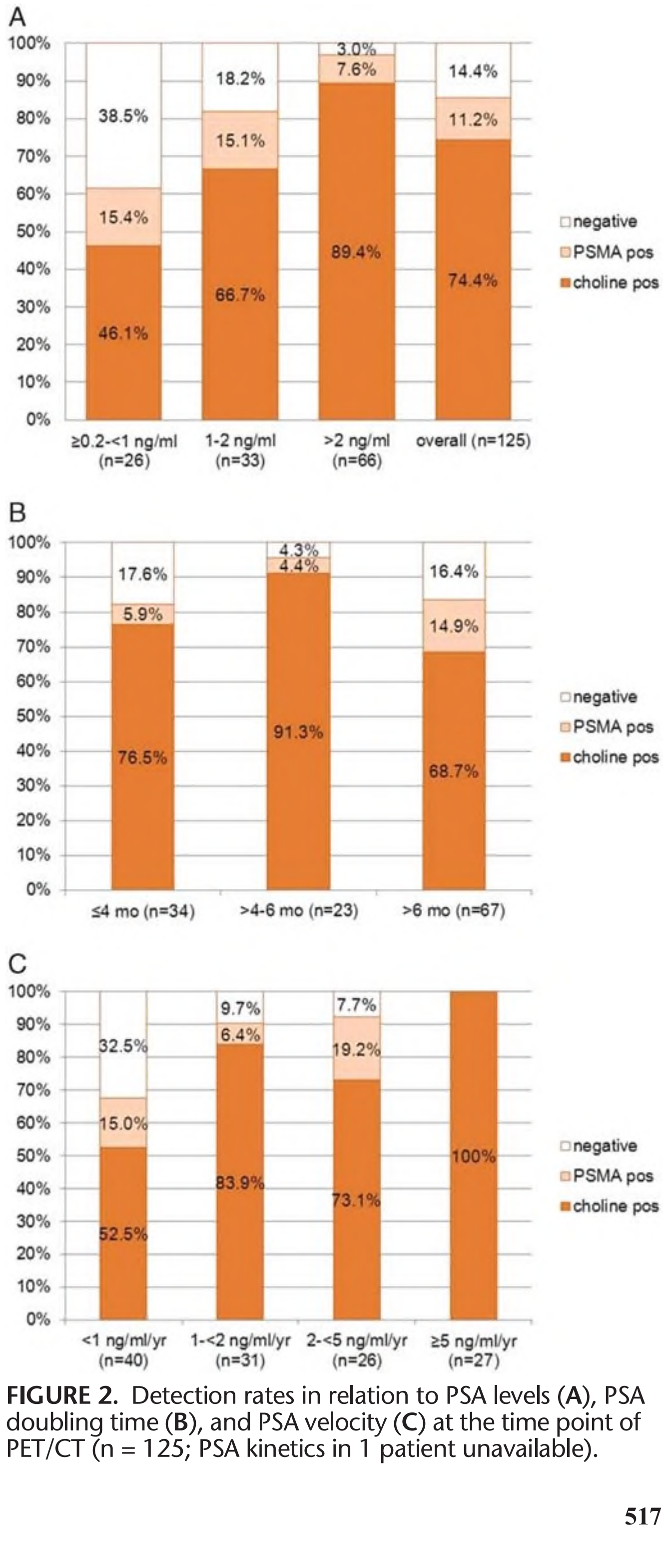

FIGURE 2. Detection rates in relation to PSA levels (**A**), PSA doubling time (**B**), and PSA velocity (**C**) at the time point of PET/CT (n = 125; PSA kinetics in 1 patient unavailable).

TABLE 2. Biochemical Findings and Lesion Detectability

	n	Sequential Imaging Approach Positive, n (%)	^{18}F-Choline Positive, n (%)	^{68}Ga-PSMA Positive, n in Choline-Negative Patients (%)
Overall	125	107/125 (85.6%)	93/125 (74.4%)	14/32 (43.8%)
PSA level (ng/mL)				
≥0.2– < 1	26	16/26 (61.5%)	12/26 (46.1%)	4/14 (28.6%)
1–2	33	27/33 (81.8%)	22/33 (66.7%)	5/11 (45.5%)
>2	66	64/66 (97.0%)	59/66 (89.4%)	5/7 (71.4%)
PSA doubling time (mo)*				
≤4	34	28/34 (82.4%)	26/34 (76.5%)	2/8 (25.0%)
>4–6	23	22/23 (95.7%)	21/23 (91.3%)	1/2 (50.0%)
>6	67	56/67 (83.6%)	46/67 (68.7%)	10/21 (47.6%)
PSA velocity (ng/mL per year)*				
<1	40	27/40 (67.5%)	21/40 (52.5%)	6/19 (31.5%)
1– < 2	31	28/31 (90.3%)	26/31 (83.9%)	2/5 (40.0%)
2– < 5	26	24/26 (92.3%)	19/26 (73.1%)	5/7 (71.4%)
≥5	27	27/27 (100%)	27/27 (100%)	—

PSA indicates prostate-specific antigen; PSMA, prostate-specific membrane antigen; *PSA kinetics not available in 1 patient;

per year (71.4%; 5/7). Further details (detection rates of ^{18}F-choline-PET/CT, ^{68}Ga-PSMA-PET/CT, and the sequential imaging approach) are depicted in Figure 2 and Table 2. The comparison of PSA levels and kinetics in the choline-positive versus choline-negative and PSMA-positive versus PSMA-negative subgroup is presented in Table 3.

Equivocal Findings

All 5 patients with equivocal findings in the ^{18}F-choline-PET/CT underwent an additional ^{68}Ga-PSMA-PET/CT. In 1 patient, ^{68}Ga-PSMA-PET/CT excluded liver and lymph node metastases confirmed using lymphadenectomy. The PSA value was stable (1.6 ng/mL postsurgery vs 1.7 ng/mL presurgery). In 2 patients with equivocal ^{18}F-choline-findings, intense focal ^{68}Ga-PSMA uptake confirmed suspicion for local recurrence and bone metastasis. The patient with bone metastases was started on antihormonal treatment. The patient with suspected local recurrence was advised to undergo salvage radiotherapy but opted for antihormonal treatment. In both patients, no follow-up serum PSA values are available.

In the remaining 2 patients, no increased ^{68}Ga-PSMA uptake was found throughout the scan, especially not in lieu of equivocal ^{18}F-choline uptake. One patient with equivocal ^{18}F-choline uptake in the prostatic fossa showed stable PSA of 0.25 ng/mL 2 months later. The other patient with equivocal uptake in a vertebral body

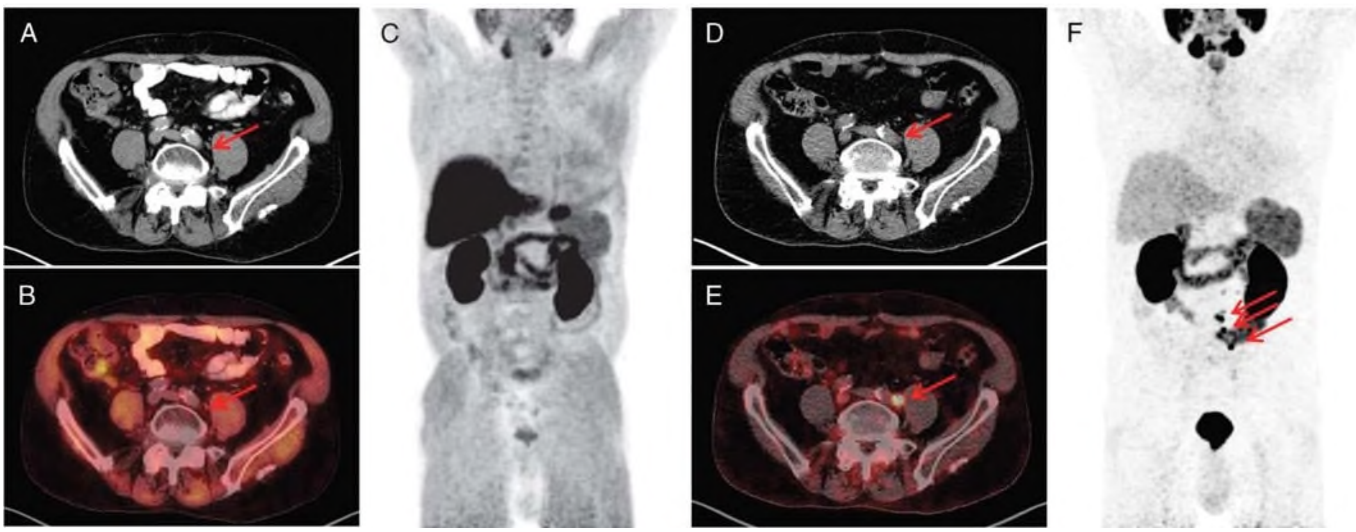

FIGURE 3. A 64-year-old patient with PSA relapse (T3aN0; Gleason score, 8; PSA level, 4.0 ng/mL; PSA doubling time, 5.9 months) radical prostatectomy, lymphadenectomy, and radiotherapy. ^{18}F-choline-PET/CT showed no suspicious lesion (**A–C**); ^{68}Ga-PSMA-PET/CT (**D–F**) showed multiple iliac and retroperitoneal lymph node metastases (arrows) without ^{18}F-choline-uptake (arrows in panel **A, B**).

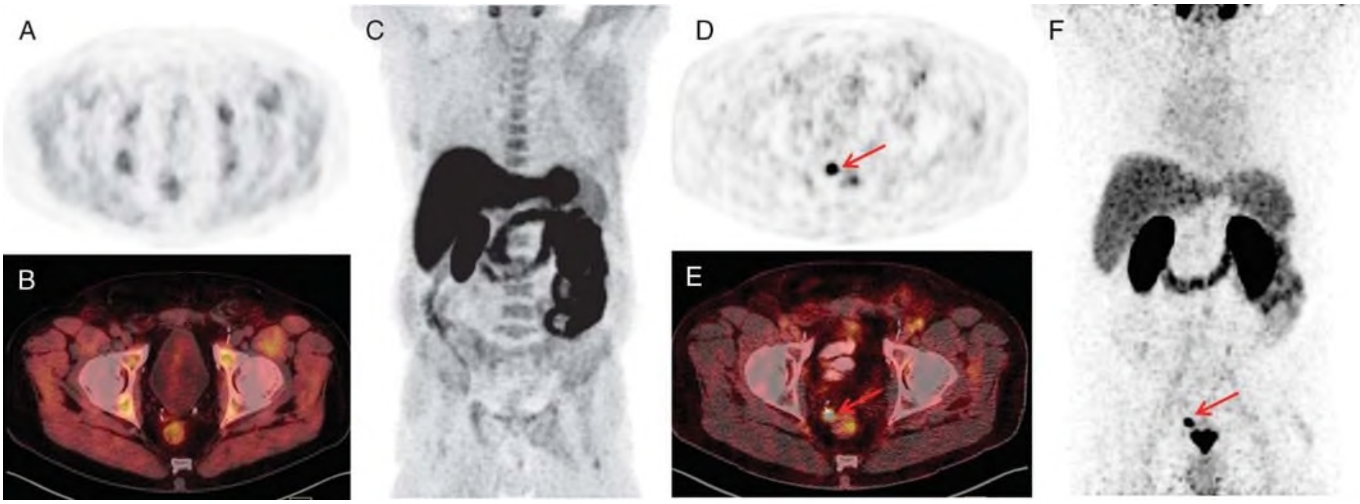

FIGURE 4. A 72-year-old patient with PSA relapse (T2cN0; Gleason score, 7; PSA level, 1.43 ng/mL; PSA doubling, 17.6 months) after radical prostatectomy and lymphadenectomy. ^{18}F-choline-PET/CT showed no suspicious lesion (**A–C**), but ^{68}Ga-PSMA-PET/CT demonstrated local recurrence (**D–F**, arrow).

(no sclerosis in CT) showed a rising PSA level (from 0.44 to 1.1 ng/mL) 4 months later; also, the follow-up ^{68}Ga-PSMA-PET/CT was read negative.

DISCUSSION

We demonstrate that ^{68}Ga-PSMA-PET/CT detects disease in 43.8% of patients with PSA recurrence but negative ^{18}F-choline scans. Adding ^{68}Ga-PSMA PET/CT in patients with negative choline scans improved the overall recurrence detection rate from 74.4% to 85.6%. The detection rate of the proposed sequential imaging approach is thus comparable to that of ^{68}Ga-PSMA-PET/CT range from 74.2% to 89.5%).[17–19,23,24] In the present cohort, additional lesions detected with ^{68}Ga-PSMA-PET/CT were located in lymph nodes and prostate bed.

The current results are consistent with ^{68}Ga-PSMA-PET/CT detection rates of 89.5% in 248 patients[19] and 82.8% in 319 patients.[18] In contrast, Ceci et al recently reported a lower detection rate of 74.2% for ^{68}Ga-PSMA-PET/CT in 70 patients with biochemical recurrence.[17] However, the inclusion criteria were different to our cohort, and in these studies, all patients underwent ^{68}Ga-PSMA-PET/CT and no sequential imaging.

The sequential imaging approach we developed and proposed detected recurrence at a higher rate than the majority of studies using C-11- (pooled detection rate of 62%, ranging from 28% to 88%)[25–28] or ^{18}F-choline (ranging from 43% to 84%).[29,30] The detection rate of ^{18}F-choline-PET/CT in the present study was consistent with these previous reports.[29,30]

The detection of recurrence is most challenging in patients with low serum PSA values. In patients with PSA values less than 1 ng/mL, the detection rate of the sequential imaging approach (61.5%) was in the range of recently published values for ^{68}Ga-PSMA-PET/CT (53% and 67%).[18,19] It was superior to the reported choline-PET/CT detection rates (19%–36% in PSA levels ≤1.5 ng/mL).[7,8,31–33]

In a preliminary study comparing ^{68}Ga-PSMA and ^{18}F-choline PET/CT in 32 patients, Afshar-Orohmieh et al demonstrated that ^{68}Ga-PSMA-PET/CT detected more lesions than ^{18}F-choline-PET/CT (78 vs 56).[23] Recently, Morigi et al confirmed these results.[34] A comparison of lesion detectability between the 2 approaches was, by design, not possible in the current study as only choline-negative patients underwent ^{68}Ga-PSMA-PET/CT. However, the clinical relevance of detecting additional lesions in patients with known metastatic disease and multiple lesions (>5) on choline-PET is debatable. In contrast, PSMA-positive findings in choline-negative patients are highly likely to impact patient management.

Recent introduction of ^{68}Ge/^{68}Ga-generators, automation of the radiopharmaceutical production, advances in peptide chemistry, and identification of relevant imaging targets have led to the successful clinical translation of ^{68}Ga-PSMA imaging.[35] However, generators provide limited activity of ^{68}Ga-labeled compounds per synthesis (1–4 patient doses depending on the generator age). The limited activity needs to be allocated to several other receptor-based imaging approaches including somatostatin and CXCR4 receptor imaging.[36] In light of the high clinical demand for

TABLE 3. Relationship Between GLEASON Score, Biochemical, and Scan Finding (n = 125 Patients; Mean (Range) and Median)

PET/CT	Choline-Positive (n = 93)	Choline-Negative (n = 32)	*P*	PSMA-Positive (n = 14)	PSMA-Negative (n = 18)	*P*
Gleason score	7 (5–10) 7	7 (6–9) 7	>0.05	7 (6–9) 7	7 (6–9) 7	>0.05
PSA level (ng/mL)	7.42 (0.22–126.59) 2.84	1.34 (0.23–4.39) 1.05	<0.001	2.01 (0.53–4.39) 1.47	1.09 (0.30–2.55) 0.74	0.054
PSA doubling time (mo)#	8.2 (0.7–51.1) 5.2	12.1 (2.1–59.6) 9.0	0.005	10.7 (2.1–19.3) 9.4	12.2 (2.4–59.6) 7.6	0.540
PSA velocity (ng/mL per year)#	10.1 (0.1–267.8) 1.4	1.0 (0.1–3.5) 0.7	<0.001	1.3 (0.2–3.5) 1.0	0.9 (0.1–2.9) 0.6	0.226

#PSA kinetics not available in one patient.

PSA indicates prostate-specific antigen; PSMA, prostate-specific membrane antigen.

peptide receptor imaging in prostate cancer patients (20–40 patients per week in many centers), the adequate supply of ^{68}Ga-activity to various imaging tests presents a clinical challenge.

In contrast, ^{18}F-labeled probes such as choline are available in near unlimited quantities. To exploit this advantage and to overcome the limited ^{68}Ga-supply, we have introduced the sequential imaging approach. An ^{18}F-choline-PET/CT study is performed first and is followed by a ^{68}Ga-PSMA-PET/CT only if the choline scan is negative. ^{18}F-choline detected sites of recurrence in 67% of all patients. Thus, the current results suggest that the need for ^{68}Ga-PSMA-PET/CT could be reduced by two thirds. This, in turn, allows for more efficient workflows and resource allocation.

The current approach is translatable and can be adopted at any imaging center. In addition, centers without access to ^{68}Ga-PSMA could perform a choline-scan first and, in case of a negative study, refer the patient to ore specialized imaging centers. A disadvantage of the current approach is the additional radiation exposure. The introduction of ^{18}F-labeled PSMA tracers[37,38] and their successful translation into the clinical routine would solve the logistical problems.[39] However, until ^{18}F-labeled PET probes are widely available, a sequential imaging approach combining choline and ^{68}Ga-PSMA tracers might be an attractive interim solution.

The current study has several limitations: First, the study group was heterogeneous and included patients after radical prostatectomy but also after other treatments with curative intent. However, the population appropriately reflects the usual clinical referral pattern. Second, this was a retrospective analysis of consecutive patients referred for PET/CT imaging. Thus, referral bias cannot be excluded. Third, subgroup analyses may be of limited value because of the small sample size. Fourth, no lesion-based analysis could be performed because, by design, ^{68}Ga-PSMA scans were only performed in choline-negative patients. Thus, it is unknown whether some ^{18}F-choline-positive lesions may have been ^{68}Ga-PSMA negative. Alternatively, ^{68}Ga-PSMA PET/CT might have revealed additional lesions. Lastly, all patients had biochemical recurrence and we calculated detection rates. However, verification of all imaging findings especially in patients with multiple lesions is practically impossible, and we accordingly refrained from calculating sensitivity or specificity.

CONCLUSIONS

The sequential imaging approach designed to limit ^{68}Ga-PSMA imaging to patients with negative choline scans resulted in comparable detection rates as reported for ^{68}Ga-PSMA PET/CT alone. Moreover, ^{68}Ga-PSMA PET/CT identified sites of recurrent disease in 43.8% of the patients with negative ^{18}F-choline PET/CT scans.

ACKNOWLEDGMENT

The authors thank Simone Seifert, Simone Groß, Michael Schulze-Glück (members of the nuclear medicine PET team), and Inge Grelle for the support and assistance.

REFERENCES

1. Uchio EM, Aslan M, Wells CK, et al. Impact of biochemical recurrence in prostate cancer among US veterans. *Arch Intern Med*. 2010;170:1390–1395.
2. Mottet N, Bellmunt J, Bolla M, et al. EAU guidelines on prostate cancer. Part II: Treatment of advanced, relapsing, and castration-resistant prostate cancer. *Eur Urol*. 2011;59:572–583.
3. Pfister D, Bolla M, Briganti A, et al. Early salvage radiotherapy following radical prostatectomy. *Eur Urol*. 2014;65:1034–1043.
4. Trock BJ, Han M, Freedland SJ, et al. Prostate cancer-specific survival following salvage radiotherapy vs observation in men with biochemical recurrence after radical prostatectomy. *JAMA*. 2008;299:2760–2769.
5. Schiavina R, Ceci F, Borghesi M, et al. The dilemma of localizing disease relapse after radical treatment for prostate cancer: which is the value of the actual imaging techniques? *Curr Radiopharm*. 2013;6:92–95.
6. Ceci F, Herrmann K, Castellucci P, et al. Impact of 11C-choline PET/CT on clinical decision making in recurrent prostate cancer: results from a retrospective two-centre trial. *Eur J Nucl Med Mol Imaging*. 2014;41:2222–2231.
7. Krause BJ, Souvatzoglou M, Tuncel M, et al. The detection rate of [11C] choline-PET/CT depends on the serum PSA-value in patients with biochemical recurrence of prostate cancer. *Eur J Nucl Med Mol Imaging*. 2008;35:18–23.
8. Castellucci P, Picchio M. 11C-choline PET/CT and PSA kinetics. *Eur J Nucl Med Mol Imaging*. 2013;40 Suppl 1: S36–40.
9. Castellucci P, Ceci F, Graziani T, et al. Early biochemical relapse after radical prostatectomy: which prostate cancer patients may benefit from a restaging 11C-Choline PET/CT scan before salvage radiation therapy? *J Nucl Med*. 2014;55:1424–1429.
10. Beer AJ, Eiber M, Souvatzoglou M, et al. Radionuclide and hybrid imaging of recurrent prostate cancer. *Lancet Oncol*. 2011;12:181–191.
11. Silver DA, Pellicer I, Fair WR, et al. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clin Cancer Res*. 1997;3:81–85.
12. Murphy GP, Elgamal AA, Su SL, et al. Current evaluation of the tissue localization and diagnostic utility of prostate specific membrane antigen. *Cancer*. 1998;83:2259–2269.
13. Bostwick DG, Pacelli A, Blute M, et al. Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. *Cancer*. 1998;82:2256–2261.
14. Ross JS, Sheehan CE, Fisher HA, et al. Correlation of primary tumor prostate-specific membrane antigen expression with disease recurrence in prostate cancer. *Clin Cancer Res*. 2003;9:6357–6362.
15. Eder M, Schafer M, Bauder-Wust U, et al. 68Ga-complex lipophilicity and the targeting property of a urea-based PSMA inhibitor for PET imaging. *Bioconjug Chem*. 2012;23:688–697.
16. Weineisen M, Simecek J, Schottelius M, et al. Synthesis and preclinical evaluation of DOTAGA-conjugated PSMA ligands for functional imaging and endoradiotherapy of prostate cancer. *EJNMMI Res*. 2014;4:63.
17. Ceci F, Uprimny C, Nilica B, et al. Ga-PSMA PET/CT for restaging recurrent prostate cancer: which factors are associated with PET/CT detection rate? *Eur J Nucl Med Mol Imaging*. vol. 42. 2015:1284–1294.
18. Afshar-Oromieh A, Avtzi E, Giesel FL, et al. The diagnostic value of PET/CT imaging with the (68)Ga-labelled PSMA ligand HBED-CC in the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging*. 2015;42:197–209.
19. Eiber M, Maurer T, Souvatzoglou M, et al. Evaluation of hybrid 68Ga-PSMA Ligand PET/CT in 248 patients with biochemical recurrence after radical prostatectomy. *J Nucl Med*. 2015;56:668–674.
20. Weineisen M, Schottelius M, Simecek J, et al. Development and first in human evaluation of PSMA I&T—A ligand for diagnostic imaging and endoradiotherapy of prostate cancer. *J Nucl Med*. 2014;55:1083.
21. Israel I, Richter D, Stritzker J, et al. PET imaging with [^{68}Ga]NOTA-RGD for prostate cancer: a comparative study with [^{18}F]fluorodeoxyglucose and [^{18}F]fluoroethylcholine. *Curr Cancer Drug Targets*. 2014;14:371–379.
22. Pound CR, Partin AW, Eisenberger MA, et al. Natural history of progression after PSA elevation following radical prostatectomy. *JAMA*. 1999;281:1591–1597.
23. Afshar-Oromieh A, Zechmann CM, Malcher A, et al. Comparison of PET imaging with a (68)Ga-labelled PSMA ligand and (18)F-choline-based PET/CT for the diagnosis of recurrent prostate cancer. *Eur J Nucl Med Mol Imaging*. 2014;41:11–20.
24. Afshar-Oromieh A, Malcher A, Eder M, et al. PET imaging with a [68Ga] gallium-labelled PSMA ligand for the diagnosis of prostate cancer: biodistribution in humans and first evaluation of tumour lesions. *Eur J Nucl Med Mol Imaging*. 2013;40:486–495.
25. Fanti S, Minozzi S, Castellucci P, et al. *PET/CT with 11C-Choline for evaluation of prostate cancer patients with biochemical recurrence: meta-analysis and critical review of available data*. vol. 43. 2016:55–69.
26. Rinnab L, Mottaghy FM, Blumstein NM, et al. Evaluation of [11C]-choline positron-emission/computed tomography in patients with increasing prostate-specific antigen levels after primary treatment for prostate cancer. *BJU Int*. 2007;100:786–793.

27. Rinnab L, Simon J, Hautmann RE, et al. [(11)C]choline PET/CT in prostate cancer patients with biochemical recurrence after radical prostatectomy. *World J Urol*. 2009;27:619–625.
28. Ceci F, Castellucci P, Graziani T, et al. 11C-choline PET/CT detects the site of relapse in the majority of prostate cancer patients showing biochemical recurrence after EBRT. *Eur J Nucl Med Mol Imaging*. 2014;41:878–886.
29. Brogsitter C, Zöphel K, Kotzerke J. 6-Choline, 11C-choline and 11C-acetate PET/CT: comparative analysis for imaging prostate cancer patients. *Eur J Nucl Med Mol Imaging* 2013;40 Suppl 1:S18–7.
30. Buchegger F, Garibotto V, Zilli T, et al. First imaging results of an intraindividual comparison of (11)C-acetate and (18)F-fluorocholine PET/CT in patients with prostate cancer at early biochemical first or second relapse after prostatectomy or radiotherapy. *Eur J Nucl Med Mol Imaging*. 2014;41:68–78.
31. Graute V, Jansen N, Ubleis C, et al. Relationship between PSA kinetics and [18 F]fluorocholine PET/CT detection rates of recurrence in patients with prostate cancer after total prostatectomy. *Eur J Nucl Med Mol Imaging*. 2012;39:271–282.
32. Castellucci P, Fuccio C, Rubello D, et al. Is there a role for ^{11}C-choline PET/CT in the early detection of metastatic disease in surgically treated prostate cancer patients with a mild PSA increase <1.5 ng/ml? *Eur J Nucl Med Mol Imaging*. 2011;38:55–63.
33. Mamede M, Ceci F, Castellucci P, et al. The role of 11C-choline PET imaging in the early detection of recurrence in surgically treated prostate cancer patients with very low PSA level <0.5 ng/mL. *Clin Nucl Med*. 2013;38: e342–345.
34. Morigi JJ, Stricker P, Van Leeuwen P, et al. Prospective comparison of 18 F-fluoromethylcholine versus 68Ga-PSMA PET/CT in prostate cancer patients who have rising PSA after curative treatment and are being considered for targeted therapy. *J Nucl Med*. 2015;56:1185–90.
35. Velikyan I. Prospective of ^{68}Ga-radiopharmaceutical development. *Theranostics*. 2013;4:47–80.
36. Herrmann K, Lapa C, Wester HJ, et al. Biodistribution and radiation dosimetry for the chemokine receptor CXCR4-targeting probe 68Ga-pentixafor. *J Nucl Med*. 2015;56:410–416.
37. Szabo Z, Mena E, Rowe SP, et al. *Mol Imaging Biol*. 2015:17–74.
38. Malik N, Baur B, Winter G, et al. Radiofluorination of PSMA-HBED via AlF chelation and biological evaluations in vitro. *Mol Imaging Biol*. 2015:17–85.
39. Dietlein M, Kobe C, Kuhnert G, et al. Comparison of [(18)F]DCFPyL and [(68)Ga]Ga-PSMA-HBED-CC for PSMA-PET Imaging in Patients with Relapsed Prostate Cancer. *Mol Imaging Biol*. 2015;17: 575–84.