Peer Review File

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Reviewer #1

In this narrative review the authors describe the recent advances of PSMA-based imaging in prostate cancer. The manuscript could be interesting for the Readers of the journal. However, I would suggest some revisions to improve the quality of the manuscript.

Comment 1: Title: "Next-generation imaging in localized and advanced prostate cancer". It does not sufficiently reflect the content of the manuscipt which is focused mainly on PSMA-based imaging that is only one of the different next-generation imaging methods for prostate cancer patients. Therefore, I would suggest to modify the title in "PSMA-based imaging in localized and advanced prostate cancer".

Reply 1: Our title was updated to “PSMA-based imaging in localized and advanced prostate cancer: a narrative review” (page 1, line 1).

Comment 2: Key words: please add the term "PSMA"

Reply 2: The key word “Prostate-Specific Membrane Antigen” was added (page 3, line 1).

Comment 3: Introduction (page 3): please add references supporting several statements in the introduction section.

Reply 3: We have added additional references on the role and limitations of conventional imaging in prostate cancer[1,2] (page 4, lines 12 and 15).

Comment 4: page 4, line 1-3: please add a reference supporting the limited role of FDG-PET in prostate cancer.

Reply 4: Two references were added.[3,4] (page 5, line 13)

Comment 5: About the diagnostic performance of radiolabeled choline, fluciclovine and PSMA-based PET, some results of single studies reported in the manuscript (in particular the less recent articles) may be substituted by more recent evidence-based data as the authors report that "we discuss the existing evidence behind the use of PSMA-targeted PET imaging in men with clinically localized, biochemically recurrent, and advanced PC." To this regard, the authors could cite this recent evidence-based summary: Diagnostic Performance of PET Imaging Using Different Radiopharmaceuticals in Prostate Cancer According to Published Meta-Analyses. Cancers. 2020;12(8):E2153.

Reply 5: Thank you for highlighting this article (published after our initial submission). We cited the above study[5] on page 6, line 15, and also included more recent meta-analyses of choline versus PSMA PET[6] (page 10, line 17) and 18F-labeled PSMA PET imaging in BCR[7] (page 10, line 8)

Comment 6: Please cite and discuss current international evidence-based guidelines about the use of PSMA-based PET for the management of prostate cancer and the suggested indications for PSMA-PET according to these guidelines.

Reply 6: Recommendations on the use of PSMA PET imaging for initial PC staging and in the setting of BCR issued by the European Association of Urology and the European Association of Nuclear Medicine and Society of Nuclear Medicine and Molecular Imaging are now discussed at the end of sections III and IV (page 9, lines 6-8 and page 11, lines 15-21).

Reviewer #2

This review article is up-to-date with essential and comprehensive information regarding PSMA PET imaging. Some points may improve the manuscript.

Comment 1: Page 2 line 7, the term "early-stage" PC seems conceptually misleading. From Section III, the reviewed papers mostly included high risk (or intermediate-to-high risk) patients to do PSMA PET for initial staging.

Reply 1: We replaced this by “clinically localized or biochemically recurrent PC” in the abstract (page 2) and avoided using this term in the rest of the manuscript.

Comment 2: Page 2 line 16, is the temporary flares of PSMA expression after initiation of therapy with sufficient evidence, this phenomenon seems was based on animal study and very limited human subjects.

Reply 2: We agree that there is limited patient data regarding PSMA flares. In the original version of the review article, we referenced 4 studies describing temporary and heterogeneous flares in PSMA uptake with initiation of hormone therapy. While the first is primarily an animal study (with only one patient),[8] the other three studies include a combined total of 32 patients.[9–11] We have added a statement describing the limitation of sample size reported to date (page 16, lines 4 and 11). However, though the sample size is limited, we still believe it is worth discussing given the potential implication for patients undergoing staging PSMA PET scans shortly after initiation of therapy (and ongoing attempts at harnessing this phenomenon to enhance response to PSMA based radioligand therapy). Slightly larger (retrospective) studies of serial PSMA in patients receiving chemo/hormonal therapy are also discussed (page 16, line 21). These studies did not report a flare phenomenon—however, longer scan intervals were used and therefore we cannot exclude the possibility of an early flare phenomenon (in a subset of patients). We have added a statement to emphasize that studies using longer scan intervals have not reported PSMA flares (page 16, line 21).

Comment 3: Page 4 line 15, it is F18-choline has high radioactivity in the urinary bladder; C11-choline has less urine secretion.

Reply 3: This was corrected to say “^{18}F-choline” in the manuscript (page 6, line 4).

Comment 4: Page 5 line 4, PSMA has significant uptake in normal human tissues, such as Kidneys, parotid and submandibular glands, duodenum, small intestines.....

Reply 4: This sentence was corrected to mention expression of PSMA by epithelial cells in the proximal renal tubules, salivary glands, small bowel and prostate gland. A reference was added (page 6, lines 18-19).

Comment 5: Page 9 line 4. The section V with only one reference 27 expressed the concept of MDT. In the following paragraph, reference 28 using choline PET/CT, indirect information from F18-DCFPyL, and PSMA-unrelated trial, these informations seemed cannot give strong support of concept of MDT.

Reply 5: We agree that evidence is still lacking to support the routine use of MDT in men with oligometastatic prostate cancer and emphasize this point in our discussion (page 12, line 4 and page 13 line 3). However, this strategy is increasingly being used in clinical practice, and is the focus of several ongoing clinical trials—therefore we believe worth discussing in this review. We updated our discussion of ongoing clinical trials at the end of this section to focus on those using PSMA PET imaging to select patients/define oligometastatic PC (page 13, lines 8-15). Furthermore, given this review's focus on PSMA-based imaging, we removed our discussion of the STOMP trial (page 12, line 15).

Comment 6: Page 14 line 14. Limitations of PSMA PET should include some pitfalls that it may also express in many benign neoplasms or non-PC malignancies.

Reply 6: We have added the following to our discussion of the limitations of PSMA PET: "[…] despite its name, PSMA expression is not specific to prostatic epithelium. Mild uptake can be seen in benign osseous conditions such as fibrous dysplasia, fractures or fibrous osseous defects. PSMA expression has also been reported on the neovasculature of several nonprostatic malignancies including renal cell carcinoma, hepatocellular carcinoma, thyroid cancers and gliomas." (page 18, lines 13-17)

Reviewer #3

In this narrative review, de Kouchkovsky and colleagues discuss the potentials and several pitfalls of prostate-specific membrane antigen (PSMA) PET imaging in various prostate cancer stages. The paper is well written and complete. Inherent to its narrative nature, it does not discuss all relevant published papers, but suffices in discussing the current state of evidence and knowledge regarding the clinical application of PSMA PET. The important gap in knowledge regarding impact of PSMA PET on clinical outcomes is recognized. I have no major concerns regarding this manuscript. I have some minor comments that the authors are asked to address.

Minors:

Comment 1: While other PET tracers (choline, fluciclovine) are mentioned, the review focusses on PSMA PET. You may want to adjust the title of the paper to include PSMA explicitly.

Reply 1: Our title was updated to "PSMA-based imaging in localized and advanced prostate cancer: a narrative review" (page 1, line 1).

Comment 2: Page 2, line 4: "increased sensitivity". Is this truly the case in primary prostate cancer?

Reply 2: The retrospective analysis by Maurer and colleagues discussed in section III showed improved sensitivity (65.9% and 43.9%) and diagnostic accuracies (88.5% and 72.3%) of PSMA PET compared to conventional imaging in men with intermediate-to-high risk disease[12] (page 7, line 16). Another retrospective analysis by Pyka and colleagues (not cited in our review) showed that Ga-PSMA PET outperformed BS for the detection of bone metastases in the primary staging and BCR setting.[13] Finally the ProPSMA trial showed higher sensitivity (85 vs 38%) compared to conventional imaging in high-risk PC[14] (page 8, line 13). Although the reference standard used in ProPSMA was a composite standard (with confirmatory pelvic node sampling in only 28% of cases) and therefore may have overestimated the sensitivity of imaging, we still believe that PSMA imaging has a higher sensitivity for the initial staging of PC than conventional imaging.

Comment 3: Page 2, line 15: comma missing after 'However …'. More missing commas like this throughout the manuscript.

Reply 3: We added the missing commas throughout the manuscript.

Comment 4: Page 4, line 16: "urinary tract uptake". Do you mean excretion?

Reply 4: We corrected this statement to say "urinary excretion" (page 6, line 5)

Comment 5: Page 4, line 22: "A subsequent meta-analysis". Please define the clinical stage (primary or BCR?)

Reply 5: The clinical stage (BCR) is included: "A subsequent meta-analysis of 9 studies and 363 patients with BCR undergoing fluciclovine imaging […]" (page 6, line 12).

Comment 6: Page 4, line 24: "is approved". By the FDA?

Reply 6: Yes; we clarified this statement to "is FDA approved." (page 6, line 13)

Comment 7: Page 5: please briefly introduce (theoretic) differences between 68Ga- and 18F-bound PSMA ligands.

Reply 7: We added a couple of sentences to discuss the difference in half-life, production and excretion of ^{68}Ga- versus ^{18}F-labelled tracers (page 7, lines 2-8).

Comment 8: Page 6: Results from the OSPREY trial are presented, referring to a conference abstract. Have these results actually been published in a peer-reviewed journal?

Reply 8: OSPREY trial results have not yet been published in a peer-reviewed journal.

Comment 9: Page 6: A recent trial on 18F-DCFPyL in primary staging has been published in EJNMMI (Jansen et al. https://link.springer.com/article/10.1007/s00259-020-04974-w). Please also discuss these results, and compare with OSPREY trial.

Reply 9: We included the SALT trial (page 8, lines 11-13).

Comment 10: Page 6: between studies, there seems to be a difference in both prevalence of lymph node metastases after dissection and patient-based sensitivity. Might this be a consequence of patient selection in terms of risk category? Please discuss your insights.

Reply 10: As suggested by the reviewer, the higher prevalence of lymph node metastases observed in ProPSMA may have been driven by the selection of a higher risk patient population (i.e. men with high-risk PC, as opposed to intermediate-to-high risk PC in Van Kalmthout et al, OSPREY and SALT). The higher patient-based sensitivity observed in ProPSMA may also be due (in part) to the composite reference standard used in ProPSMA with only 28% of patients undergoing confirmatory pelvic node sampling (page 8, line 16). We have made sure to highlight both of these nuances in our discussion of ProPMSA (page 8 lines 20-23).

Comment 11: Page 6, line 24: "These results led to a management change in 27% of cases.". Please provide more information on what kind of management change.

Reply 11: This refers to high or medium impact changes in management, which were defined as change in treatment intent, modality, extent of surgery, and RT dose or volume (page 9, lines 3-4)

Comment 12: Page 7, line 14: "per-patient sensitivity and PPV of 99%". I wonder how valid this is in BCR setting. Did these studies mainly biopsy PSMA-positive lesions? In that case this sensitivity might be an overestimation.

Reply 12: Indeed, most of the 15 studies included in this meta-analysis were retrospective in nature and often used results of pre-operative ^{68}Ga-PSMA imaging to guide the extent of lymph node dissection (or only pursued pathologic confirmation in patients with positive ^{68}Ga-PSMA PET scans). We added this point to our discussion, emphasizing that this may have led to an overestimation of the sensitivity of Ga-PSMA in BCR (page 9, lines 19-22).

Comment 13: Page 12: In some studies, patients with PSMA-/FDG+ disease were excluded (e.g. http://jnm.snmjournals.org/content/early/2019/11/21/jnumed.119.236414.full.pdf). Perhaps you could comment on this rationale?

Reply 13: This is discussed in the following paragraph: Several studies have investigated the relationship between pre-treatment ^{68}Ga-PSMA PET uptake and treatment outcomes in patients receiving ^{177}Lu RLT: in one analysis of 30 patients with mCRPC, whole body tumor SUV_{mean} on pre-treatment ^{68}Ga-PSMA PET imaging predicted absorbed radiation dose on post-treatment quantitative SPECT/CT,[15] which was in turn associated with PSA response at 12 weeks. In another phase 2 trial, pre-treatment PSMA SUV was predictive of ≥30% PSA reduction in patients receiving ^{177}Lu RLT.[16] Grubmuller and colleagues also showed that on-treatment changes in ^{68}Ga-PSMA PET total tumor volume were predictive of PSA response and overall survival. Together these finding provide a rational for the use of ^{68}Ga-PSMA PET/CT to select patients for PSMA-targeted therapies, and those with low PSMA uptake (generally defined as a tumor SUV_{max} less than 1 to 1.5 times that of the liver) have been excluded from clinical trials of PSMA RLT. Yet while patients with high PSMA uptake may derive the most benefit from PSMA-targeted therapy, the efficacy of RLT in an unselected patients population—or among patients with low PSMA expression—has not been fully investigated (page 15, lines 7-19).

Comment 14: Page 13: I am missing some data on the use of PSMA in monitoring mCRPC treatment with chemo/hormonal therapy. Have you found data on this?

Reply 14: Data on the use of PSMA PET as a biomarker of treatment response is still limited. For mCRPC patients treated with chemo/hormonal therapy we included (1) a retrospective analysis of 43 mCRPC patients undergoing 67 systemic therapies (radium-223 n=9, cabazitaxel n=12, docetaxel n=22, abiraterone n=6, and enzalutamide n=18), which showed a significant correlation between changes in PSMA PET parameters (e.g. total tumor volume, SUVmax, SUVmean)[18] and PSA response to therapy and (2) another retrospective analysis of 26 mCRPC patients started on abiraterone or enzalutamide, which found a perfect association between on-treatment decreased PSMA uptake and PSA or radiographic response[19] (page 16, lines 23-24 and page 17, lines 1-9).

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