Peer-Review comments and authors responses

"Prostate-Specific Antigen Screening and Overdiagnosis in Prostate Cancer: A Systematic Review and Meta-Analysis"

Dear Reviewers:

We would like to express our sincere gratitude for the time and effort you dedicated to reviewing our manuscript entitled "PSA Testing and Overdiagnosis in Prostate Cancer: A Systematic Review and Meta-Analysis" We appreciate your comments, for they have improved the quality and clarity of our work. Below, we provide a detailed response to each of your remarks. Please do not hesitate to let us know should any aspect require further clarification or modification.

Reviewer 1

METHODS

1. **Comment:** Search strategy and study selection. There is inconsistency in reporting the number of included studies: the Abstract states "Eleven out of 13 identified studies were included," Methods/Results state "13 studies met the inclusion criteria," and Table 1 lists 12 studies. This should be verified and clearly reported.

Response: *The inconsistency was checked and we identified the paper that was missing in Table 1. This work was added to the table (please see Table 1, page 35).*

2. **Comment:** Search strategy issues: Table S1 shows inconsistent syntax (mix of MeSH and title/abstract phrases). The use of terms such as "40 years and older"[tiab], "comparative study"[tiab], and "observational study"[tiab] without established PubMed filters (e.g., MeSH terms) likely limited retrieval of eligible studies. Please justify this approach and discuss its impact as a potential limitation.

Response: *we recognized this as a limitation. "A key limitation of this review is the use of a restricted search strategy with the combination of MESH terms and Titles and abstract phrases, which could have limited the number of studies included in this review." (Line 20-22 limitations Section, page 14).*

3. **Comment:** Embase and Cochrane CENTRAL were not searched. These databases frequently index RCTs not available in PubMed. Please justify their omission.

Response: *We unfortunately did not have access to them, therefore, we did not use them in this article.*

4. **Comment:** Clarify whether the review protocol was pre-registered (e.g., PROSPERO ID). If not, explain why.

Response: *We thank the reviewer for this important observation. The review protocol was not pre-registered in PROSPERO due to the exploratory nature of the project and institutional time constraints at the time of initiation. We also posed this question to the journal´s editor and the answer that it was not required in the PPCR Journal.*

5. **Comment:** "Overdiagnosis" is not a uniform concept. It can refer to excess incidence, lead-time adjusted models, or pathology-based definitions, each producing different results. The manuscript reports widely varying relative risks but does not clearly explain how "overdiagnosis" was defined in each included study or how differences were harmonized for meta-analysis. Please explicitly define "overdiagnosis" for each included study. Clarify whether subgroup analyses were performed for different definitions or study types. Discuss the methodological complexity and variability of overdiagnosis in the Discussion.

Response: *We defined overdiagnosis and included it in the Methodology Section. "For this study, overdiagnosis was defined as non–clinically significant prostate cancer, characterized by a Gleason score <7 or ISUP grade 1 at diagnosis, or as cancer unlikely to cause harm or be detected within the patient's lifetime. This definition aligns with those adopted by the included studies, when specified. "(Methodology, Data Synthesis, lines 21-25, page 6). No subgroup analysis was performed.*

6. **Comment:** Eligibility criteria are unclear. The Methods section states inclusion of "descriptive studies, randomized controlled trials, and observational studies" but excludes " non comparative designs." Clarify what is meant by "descriptive studies" and how such studies contribute to pooled effect estimates. Consider refining terminology to avoid confusion (e.g., remove "descriptive" if not applicable).

Response: *We modified the terminology to improve clarity. "The article's search included published randomized clinical trials (RCTs) and observational studies (cohort) in English for the diagnosis of prostate cancer using PSA screening compared to no screening (placebo or standard care). Details of the search strategy are outlined in Table S1. "(METHODOLOGY, Information Sources and Search Strategy, line 13-16, page 5)*

RESULTS

7. **Comment:** Explicitly state the final number of included studies and reasons for exclusion (e.g., "Of 576 identified records, 13 studies met inclusion criteria. Main reasons for exclusion were lack of relevant outcomes (n=X), inappropriate design (n=Y), etc.").

Response: *We stated the reasons for exclusion of the papers. "The initial search identified 576 studies, which were imported into Covidence for screening. After the removal of 8 duplicates*

manually and an additional 161 duplicates automatically identified by the software, 407 unique records remained for title and abstract screening. Following this stage, 215 studies were excluded as irrelevant, leaving 192 studies for full-text assessment. Of these, 179 were excluded for the following main reasons: wrong patient population (n=47), inappropriate study design (n=34), irrelevant outcomes (n=32), wrong intervention (n=28), and unsuitable comparator (n=18). Ultimately, 13 studies met the inclusion criteria and were included in the final synthesis. However, the study by Krilaviciute et al. (2023), although included in the systematic review, was excluded from the quantitative analysis, as no other study compared digital rectal examination (DRE) to prostate-specific antigen (PSA) testing. " (Characteristics of the studies, line 29, pages 7-8).

8. **Comment:** Provide a structured summary of study populations: e.g., "Of the 13 included studies, X were RCTs (total N = xx, median follow-up xx years), with the largest trial (CAP, >400,000 men) contributing xx% of total sample size. Most studies were conducted in Europe (n=8), two in North America, one in Asia." Clarify population/sample size ranges and contributions to pooled analysis.

Response: *We revised the manuscript and clarified: "Sample sizes ranged from 4,276 to over 400,000 participants, with most trials using randomized controlled designs. Of the 13 studies included, 12 were Randomized Controlled Trials, with a total number of participants N: 1149808, median follow-up time: 16 years, and two of the papers included, due to their set outcome, did not follow up the patients. Out of this, the one by Martin, R.M. et al. was the largest one, accounting for 36.12% of the total population. All studies were conducted in Europe; only one included data from the United States of America." (Population, lines 11-16, page 8)*

9. **Comment:** The review notes that newer trials using MRI/biomarkers report lower overdiagnosis and complications but does not present formal subgroup analyses. Consider stratifying results by trial era (pre- vs post-2020) or by methodology (MRI/biomarker vs traditional PSA).

Response: *We ran a subgroup analysis with the studies published after 2020 and it did not show difference with the overall analysis. This was included as a Forest. (Figure 2 Legend, lines 12-13, page 45).*

DISCUSSION

10. **Comment:** The Discussion currently reads as a large block of information. Consider structuring it into clear thematic subsections, such as:

- Key findings and their interpretation
- Overdiagnosis and overtreatment
- Heterogeneity and generalizability
- Policy implications
- Limitations and strengths
- Future directions

Response: *we did the changes suggested and separated the results, so it would be easier and clearer for the reader.*

11. **Comment:** You state: *"PSA screening can reduce prostate cancer-specific mortality, especially in younger populations undergoing repeated testing."* But later also note: *"No significant difference in mortality after 15 years" in Martin et al. (2024).*" There is a tension here that should be discussed more explicitly: under what conditions does PSA screening reduce mortality, and why might some large studies fail to show this? This could be due to differences in study design, screening intervals, population risk profiles, or length of follow-up. Expanding on this strengthens your interpretation

Response: *We included in the discussion that the Martin et al. (2024) study was a secondary analysis study with 20 times the number of participants than Hugosson et al. (2018) and Franlund et al. (2022). "Studies from Latin America (Tourinho-Barbosa et al., 2016) reflect barriers to prostate cancer screening, reflecting global disparities. Similarly, the UK trial by Martin et al. (2024), with over 400,000 participants exceeding the cohorts of Hugosson et al. (2018) and Franlund et al. (2022), found no mortality difference after 15 years, reinforcing the discussion on the long-term effectiveness of population-wide PSA screening. "(Discussion, Overdiagnosis and overtreatment, lines 7- 11, page 11).*

12. **Comment:** You reference percentages (20–50% of cancers detected via PSA are insignificant). However, it would be useful to contextualise this range: is it consistent across all studies reviewed? Does this vary by age group, screening frequency, or region? Suggest explicitly discussing the clinical consequences of overtreatment (e.g., impact on quality of life, psychological distress, and healthcare costs.)

Response: *Although overtreatment is relevant, it is beyond the scope of our study to explore its consequences in other spheres of the patient´s life, therefore this was not analyzed nor discussed.*

13. **Comment:** Your limitation section is good but could be expanded. You mention search strategy limitations. Did you also consider publication bias, language bias, or exclusion of grey literature? Could heterogeneity in study design and outcome definitions itself limit synthesis?

Response: *we expanded the limitations section as suggested: " A key limitation of this review is the use of a restricted search strategy with the combination of MESH terms and Titles and abstract phrases, which could have limited the number of studies included in this review. Embase and Cochrane CENTRAL were not included due to the lack of institutional access, and only open-access databases were used. Our exclusion and inclusion criteria only allowed for studies that have both PSA screening and no PSA screening present at the same time in each paper, which could have limited the number of articles included and Our search was limited to major open access databases to prioritize high quality articles. We acknowledge that this could lead to*

publication bias and , because those that only show PSA screening complications could have been missed out, which could have explained the low numbers of complications reported in this review." (Limitations and Strengths, lines 20-25, page 14).

OTHERS

14. **Comment:** The manuscript states use of ChatGPT for drafting/rephrasing. Expand this statement to specify: Which sections were AI-assisted.mWho verified references and results. Please confirm that no data were fabricated/generated by AI.

Response: *We acknowledge the use of AI to improve the English language and references structure: ChatGPT. Optimizing language models for dialogue. https://openai.com/blog/chatgpt/; n.d. Accessed June 25, 2025. We did not fabricate nor generate data using AI, the ideas written in the paper represent the author´s.*

Minor Comments

- Ensure consistent use of past tense for study results and present tense for interpretations and conclusions
- Avoid redundancy (e.g., "Figure 2 summarizes the forest plot" can be integrated into text).
- Use consistent terminology ("screening group" vs "intervention group").
- Avoid repetition: some heterogeneity points appear twice.
- Pleas cite guideline recommendations (e.g., when discussing USPSTF, AUA, EAU guidelines).

Response: *the use of past tense, redundancies, consistent terminology and guidelines citations were all addressed.*

Reviewer 2

ABSTRACT

1. **Comment** – Please check, the phrase "were searched" is repeated in the Methods.

Response: *the "were searched" repetition was erased.*

ABBREVIATIONS

2. **Comment** – "Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Ensure that there is a consistent pattern of abbreviations throughout the article. Superscript Arabic numerals are used for such footnotes." https://journal.ppcr.org/index.php/ppcrjournal/authors-center

Response: *the missing abbreviations were added in the first page and underneath Table 1.*

METHODS

3. **Comment:** Please revise the search strategy and inclusion criteria items. In the search strategy authors declare they included published randomized clinical trials (RCTs) while in the inclusion criteria, observational studies and other study designs were included.

Response: *This was addressed in the Search Strategy section, line 14, page 5.*

RESULTS

4. **Comment** – Please provide legends and define the abbreviations for the tables.

Response: *the missing abbreviations were added in the first page and underneath Table 1.*

DISCUSSION

5. **Comment** Please define the abbreviations USPSTF and AUA/SUO.

Response: the definitions for both abbreviations were defined in page 1.

6. **Comment** Please provide the reference for the following statement "Simulation models estimate a 13–20% increase *in mortality if screening is completely discontinued."*

Response: *We added the reference for that statement Gulati R, Tsodikov A, Etzioni R, Hunter-Merrill RA, Gore JL, Mariotto AB, Cooperberg MR. Expected population impacts of discontinued prostate-specific antigen screening. Cancer. 2014 Nov 15;120(22):3519-26. doi: 10.1002/cncr.28932. Epub 2014 Jul 25. PMID: 25065910; PMCID: PMC4221407.*

7. **Comment** In "The Latin American panorama highlights the urgent need to optimize prostate cancer detection, with over 230,000 new cases annually and high mortality in countries like Mexico, Brazil, and Colombia (Pérez-Ramírez et al., 2023). This review aims to guide screening strategies that reduce mortality while minimizing harms related to PSA screening." the authors provide important epidemiological data on prostate cancer in Latin America and at the same time describe that the study is aimed at guiding screening strategies. The way that this paragraph is structured implies that the study is restricted to Latin America populations. Please clarify this information, considering that the study aim was stated before as follows: "to evaluate whether PSA-based screening for prostate cancer, especially when compared to risk-adapted strategies (e.g., mpMRI, biomarkers, risk calculators) or no screening, reduces overdiagnosis and improves clinically relevant outcomes."

Response: *we rephrased the paragraph to improve clarity: "Studies from Latin America (Tourinho-Barbosa et al., 2016) reflect barriers to prostate cancer screening, reflecting on global disparities. Similarly, the UK trial by Martin et al. (2024), with over 400,000 participants exceeding the cohorts of Hugosson et al. (2018) and Franlund et al. (2022) found no mortality difference after 15 years, reinforcing the discussion on long-term effectiveness of population-wide PSA screening." (Discussion, Overdiagnosis and overtreatment, lines 6-10, page 11).*

8. **Comment** KEY POINTS - Please consider rephrasing this sentence: "Screening keeps most men alive just as long." Suggestion: Screening does not extend the lifespan of men.

Response: *This has been rephrased to the following: Screening has not been shown to prolong men's lifespan (Keypoints, line 1, page 15).*

9. **Comment** This systematic review and meta-analysis study provided relevant data on the subject of prostate cancer and PSA screening. However, it is important to take into account this study's limitations. Given the high heterogeneity (I^2) and the results shown in the sensitivity analysis, the authors should be more cautious with statements that intend to guide screening strategies and possibly change health policies on prostate cancer.

Response: *The previous comment has been addressed: " This review aims to provide an insight into PSA screening strategies that might reduce mortality while minimizing harms related to PSA screening. Due to the high heterogeneity observed across studies and sensitivity analysis, findings should be interpreted with caution" (Discussion, Overdiagnosis and overtreatment, lines 23-26, page 11).*

OTHERS

10. **Comment** FORMATTING – The manuscript should be formatted according to PPCR Journal guidelines. Please check https://journal.ppcr.org/index.php/ppcrjournal/authors-center

Response: *We formatted the manuscript according to the PPCR Journal guidelines.*

Reviewer 3

ABSTRACT

1. **Comment** Please organize your abstract by Background, Methods, Results and Conclusion, and Fix typos: "PubMed, Web of Science, and Scopus (2015 onward) were searched. were searched."

Response: *the Abstract was organized as suggested, the typing errors were corrected and the repetition of "were searched" was erased.*

METHODS

2. **Comment** Fix typo: "**Figure 1** summarises the Prisma flow diagram."

Response: *the typing error was corrected.*

3. **Comment** Page 7. Population: "Sample sizes ranged from 4,276 to over 400,000 participants, with most trials using randomized controlled designs." Provide more details about the population such as Country, gender/biological sex percentage, and average age.

Response: *This has been changed to: Of the 13 studies included 12 were Randomized Controlled Trials, with a total number of participants N: 1149808, median follow up time: 16 years and two of the papers included, due to their set outcome, did not follow up the patients. Out of this the one by Martin, R.M. et al. was the largest one, accounting for 36.12% of the total population. All studies were conducted in Europe, only one included data from the United States of America. (results section, Population, lines 12-16, page 8).*

DISCUSSION

4. **Comment** Based on the results of the Funnel plot, authors should include the publication bias risks in the discussion and highlight a conservative interpretation of the results.

Response: *This was addressed as follows: "Our search was limited to major open access databases to prioritize high quality articles. We acknowledge that this could lead to publication bias and, because those that only show PSA screening complications could have been missed out, which could have explained the low numbers of complications reported in this review. On the other hand, the strengths of this review are the large sample size, which adds strength to the generalizability of the results."(Outcomes, Limitations and strengths, lines 23-27, pages 14).*

5. **Comment** More typos have been observed along the text, please fix it: "tumours", "personalised"

Response: *the mentioned mistakes have been corrected.*

6. **Comment** No contractions in academic writing such as "wouldn't". Please fix those.

Response*: the changes have been made as suggested.*

Reviewer 4**:**

4. Abstract section:

1. **Comment:** The phrase *"were searched"* in the "Methods" section is repeated; rephrasing would enhance the flow of this section.

Response: *The repetition of "were searched" has been erased.*

5. Conclusions section:

2. Comment: The tone of the conclusion currently reads as somewhat informal. Adjusting the wording to more formal scientific language would help align it with the style of the rest of the manuscript.

Response: *The conclusions have been rewritten to a formal style: "Our findings indicate that the careful implementation of PSA screening may not result in a substantial increase in overdiagnosis. A modest reduction in prostate cancer–specific mortality was observed, with no relevant change in overall mortality and no significant rise in diagnostic pathway–related complications. We suggest that future investigations focus on optimizing risk-stratification strategies to more precisely identify individuals most likely to benefit from PSA screening while minimizing associated harms." (Conclusions section, lines 19-23, page 15)*

OTHERS

3. Comments - Figure 1 (PRISMA Flow Chart):

-In the section *"references from other sources"*, the value is reported as *n = ?*. Please clarify or consider excluding this section if not applicable.

-In the section *"references removed"*, the category *other reasons n = ?* should be specified; if none exist, it may be clearer to state *n = 0*.

-In the section *"studies assessed for eligibility"*, there appears to be an additional line under *"wrong patient population"*. Currently, only 171 exclusions are justified, and 8 studies remain excluded without justification. Please revise to ensure consistency and transparency.

Response: *Figure 1 has been reviewed and the changes suggested were made.*

4. Comments - Figure 5 (Risk of Bias and NOS tools):

-The figure appears distortional and would benefit from improved formatting.

-For the NOS assessment, results could be represented according to the number of stars attributed to each domain.

-For the RoB2 assessment, please clarify how the overall risk of bias is determined. Typically, if any single domain is rated as high risk, the overall assessment should also be classified as high risk.

Response: *The RoB2 assessment has been clarified and the configuration of the images has been improved.*

5. Comment - Table 1 (Characteristics of Included Studies):

The results presented in this table could be summarized more concisely, perhaps into 2–3 lines, to improve readability.

Response: *Table 1 has been summarized as suggested.*

We appreciate your constructive and thoughtful feedback,

Tania González, MD. MSc.
On behalf of all authors