Peer Review File

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Reviewer A

Excellent study of a long overdue update on pain scales for transperineal biopsy in more recent years with more advanced knowledge of local anesthesia techniques for this approach.

Please note several important recommended changes:

1. The title needs to specify that this local anesthesia biopsy was done with the aid of the KOELIS Full Grid™ device; since many different skin punctures are required with a grid, you must distinguish this study from fusion biopsies using a transcutaneous cannula like the PrecisionPoint device, where only two skin punctures are required.

Reply 1: This is a very important remark, thank you. (Also, please note, that in the previous version of the manuscript we erroneously wrote that we used "Full Grid" version of the device, while we actually used the "Mini Grid". We apologize for this mistake. From our point of view, this is very unlikely to impact the interpretation of the results.

Changes in text: In the Title, we changed "transperineal" to "grid-based transperineal". Also, in the Methods we corrected Full Grid to Mini Grid (Line 75).

2. You should use the updated Wong-Baker Faces Pain Rating Scale which doubles your numbers from 0-5, to 0-10, so that your numbers can be compared to pain ratings generally used in the literature.

Reply 2: Thank you very much for this suggestion. This modification will indeed improve the recognizability of our results, as well as allow for direct comparison with other studies.

Changes in text: In the manuscript and in the Figure 1, we doubled all the numbers that referred to the pain rating scale. In the Methods, we mentioned that the original picture consisted of a 0-5 scale, but we doubled the scores for the purpose of this study. Also, in the Discussion we revised the considerations that regarded the inequality of the pain scores. (Multiple changes throughout the manuscript).

3. If possible, please describe in more detail how pain scores were reported by the patient:

Were patients shown the Wong-Baker FACES Pain Rating diagram by a nurse, by a physician? By the physician who did the biopsy or another physician?

What question was posed in asking the patient to report his pain rating?

What was your general estimation of pain of the whole procedure?

What was the worse pain you experienced during the procedure?

Was the pain you are rating from the introduction of the transrectal probe, the injection of anesthesia or during the actual biopsy?

Reply 3: This is a very important issue, thank you very much for raising it. The diagram was shown by the performing urologist, right after the end of the procedure, and while there was no protocol for asking a specific question, patients were asked to report their general, overall experience.

Changes in text: We rewrote the "Pain reporting" paragraph to include the above details. (Lines 110-116).

4. Please mention the maximum lidocaine dose accepted by the WHO in the methods (**4.**5 mg/kg)
Reply 4: Thank you for this important remark.

Changes in text: We added a following sentence into the methods: "The total lidocaine dose was 175-200 mg and never exceeded the World Health Organization recommended maximum dose of 4.5 mg/kg or 300 mg" (Lines 99-101)

5.re: using different pain ratings than other publications:

It seems easy to modify your pain scores by simply doubling them as you are using the same Wong Baker Faces Pain Rating pictures used by what others report in the literature; you can state you are doubling the pain scores in the methods section.

Reply 5: Thank you for raising this issue. We have replied in our reply 2.

Changes in text: As previously stated, we adopted multiple changes to the manuscript and revised Discussion, so that this issue is no longer considered a limitation. Thank you.

6. I've attached a pdf with some grammatical and typo corrections.

Reply 6: We greatly and truly appreciate this large effort of yours to improve the quality of our manuscript. Please, accept our deepest thanks.

Changes in text: We implemented vast majority of the suggested revisions into the text. Thank you. We did not delete the sentence fund in Lines 77-79 in your PDF file, as it explains the rationale of our risk factor analyses and is necessary for the STROBE checklist. (Multiple changes in the text).

Reviewer B

In general, it's an interesting and actual topic as the TP should be preferred. For wider acceptance performance under LA is also mandatory!

However, a few minor corrections/suggestions should be considered:

Page 2, line 46: Please write out numbers from 1 – 10

Reply 1: Thank you very much for this remark. We are aware that according to most editorial rules, numbers from 1 to 10 should be expressed as words, however, while those particular numbers are used to describe a numerical scale, we believe that leaving the digits would provide better visualization of the tool that was used. Moreover, most of the cited studies also use digits when describing the pain score.

Changes in text: None.

Page 3, line 70: […]. This approach …[…] Page 4, line 91: Data were collected

Reply 2: Thank you for your suggestions, we corrected the text accordingly.

Changes in text: As above.

Page 5, line 106: see 1.

Please, see Reply 1.

Page 5, line 107 ff.: Please define and describe how many systematic cores were taken. Do you have a specific standard? 12-core template?

Reply 3: This is a very important question, as the issue of using a template or not would be strictly regarded to the length or extent of the procedure. Thank you for rising this topic. In our institutions we do not use a specific template. The number and distribution of systematic cores is dependent on the performing urologist decision. The systematic cores are aimed not to overlap with the targeted cores. The number is dependent on the size of the prostate, usually does not exceed 8. Also, in cases of large targeted lesions, the number of systematic cores would be lower, not to overlap with targeted cores.

Changes in text: The sentence: "No specific template for systematic biopsy was used" was added in the Methods section, before the sentence: "The number and distribution of systematic cores were at the discretion of the performing urologist, dependent predominantly on the lesion location and size, as well as prostate volume (PV)" (Line 84 and following)

Local anesthesia procedure: Why did you use 1 % lidocaine solution for the subcutaneous superficial injection and a 0.5 % lidocaine solution for the deeper injection? Any reasonable explanation?

Reply 4: Thank you very much for this comment. Due to paucity of research papers describing exact LA procedures that result in good anesthesia, we could not adopt any evidence-based protocol and thus developed our institutional LA protocol. There is no other reasonable explanation for this specific technique. In fact, this is a technique that we were taught by a mentor a few years ago. Using low-concentration (as compared to 2%) solutions allow for wider distribution of the agent without exceeding maximum total dose.

Changes in text: None required.

Furthermore, you should describe if any kind of antibiotic propyhlaxis was admininstered. Of course, this was not part of your study's focus. But there is an ongoing debate about the need or the avoidance of antibiotic prophylaxis in TP procedures.

Reply 5: This is an important remark. Most of the patients received preprocedural antibiotic prophylaxis consisting of a single dose of either 400 mg cefixime or 960 trimethoprim/sulfamethoxazole, administered 2 hours prior to the biopsy.

Changes in text: We added this information to the manuscript (Lines: 87-89).

6. Page 8, line 179: What do you mean with largest studies? Largest of what? TP procedures in LA? Please specify this more in detail

Reply 6: This comment is very accurate and thank you for that. Yes, you are right that we should not have said it this way. It is hard to put our study into a single category and assess its position in terms of sample size.

Changes in text: We rewrote the sentence so that it does not contain words comparing our paper to other studies in regard to sample size (Lines 173-174).

Page 9, line 201 ff: Taking these findings into consideration …is methodologically correct (I would just remove this sentence. Is too long and not really relevant

Reply 7: Thank you very much for this valuable suggestion.

Changes in text: We deleted the sentence.

Page 9, line 206: .. that the vast majority …

Page 10, line 240 ff.: "Despite one could assume .." (please change this sentence, e.g. We assume that patients … but our data show that…

Page 12, line 285-289. : You should definitely shorten this sentence. Please use more full stops instead of commas.

Reply 8: Thank you very much for the above suggestions aimed to improve the quality of the text.

Changes in text: We revised the text accordingly.

Please revise you Result part: Here you should mention all the relevant results and information. For example you have mentioned the AFS aspect in the Discussion part. This belongs into the Result Section.

Reply 9: Thank you for this comment. Referring to results in our Discussion section may seem confusing. However, our Result section already contains all the results, either mentioned in the text, or in the tables. In this particular example, the data on the AFS aspect are presented in Table 1 (Patient characteristics), Table 2 (Comparison of AFS rates between SP and non-SP patients, no significant difference demonstrated), Table 3 (AFS as a factor in a univariable model, no significant association with SP). We discussed it broadly in the Discussion section mainly to explain why we considered the AFS location as a factor and to discuss the role of lesion location in regard to pain with penetration by the biopsy needle. For clarity and according to editorial rules, data included in tables is not narratively presented in the Results.

Changes in text: None.

Reviewer C

I would like to thank the authors for this manuscript which covers an important topic regarding the current transition towards transperineal prostate biopsy and the, at the same time, high financial pressure on the healthcare system and quality of life issues within early prostate cancer detection. The authors of this manuscript aim to i) investigate (retrospectively) the tolerability in terms of pain of transperineal prostate biopsy under local anesthesia and ii) to identify risk factors for significant pain experience. The median pain score was low and younger age was associated with significant pain experience. The authors translate their results to clinical practice by opting for more extensive anesthesia in younger patients which makes this study interesting for the readers of TAU. However, I have several minor and major comments and suggestions which might further improve the manuscript to make it suitable for publication.

Abstract:

- Line 48: Please report IQR.

- Report percentages to two significant figures (line 49).

Reply 1: Thank you for these valuable remarks.

Changes in text: We corrected the Abstract accordingly.

Introduction section:

Line 80: please mention explicitly that it is about transperineal biopsy since MRI/US fusion biopsy can also be performed rectally. **Reply 2:** Thank you very much for noticing this.

Changes in text: We corrected the sentence accordingly (Line 54).

Materials and Methods section:

Line 122: was 15-20 mL lidocaine injected on each side, so in total 30-40 mL or 15-20 mL divided over two sides? Please specify.

Reply 3: We admit that the original sentence might have been confusing, thank you for raising our attention to this. 15-20 lidocaine was injected on each side (30-40 mL in total). We rephrased the sentence in the revised version of the manuscript.

Changes in text: The sentence is now: "with 15-20 mL of solution being injected on each side (larger amounts with larger PV)" (Line 99).

A crucial aspect of the pain score is how this is interpreted by the patient (as discussed by the authors later in the manuscript). So how was this asked: As an overall score (including lidocaine injection and biopsy cores) or was it asked for the biopsy cores only (so after the lidocaine injection)? Could the authors elaborate on this in the method section?

Reply 4: Thank you for this very important comment. Immediately after the procedure (after the patient left the table) a pain score diagram was shown to the patient by the performing urologist and the patient was asked to pick their overall experience.

Changes in text: We revised this subsection of the Methods (Lines 110-116).

Result section:

- Please report reasons for exclusion. How many patients did not report their pain score/missing values.

Reply 5: Thank you for your comment. Firstly, we would like to explain and apologize that initially we provided slightly wrong data in regard to the patient selection process. We wrote that 460 patients were identified and that pain reports were available for 300 patients, and one was further excluded due to missing data. This is not true, and we are verry sorry for this error. The number of patients who were identified were 459, the number of patients for whom pain reports were available was 299 and none of these patients was further excluded due to missing data. The person responsible for writing this paragraph erroneously included one patient who underwent transrectal biopsy into this count and then wrote that the man was excluded due to missing data (in fact, this patient was never included into the analyses, so this error did not influence the results in any way).

We now verified this and corrected. To answer your question, as stated in the results section, we identified 459 patients who met the inclusion criteria (i.e., patients who underwent TP biopsy under LA, with positive MRI), and then we excluded 160 patients who did not report their pain. The explanation to this data gap (no pain reports) is difficult to provide on a retrospective basis. Most probably not every patient was asked to report their pain. Deeper analysis of our database shows, that pain was being assessed in every patient since January 2021. However, before this date, we have data gaps in records related to biopsies performed on particular days. Most probably the diagrams were not available in the office on those particular days (we use printed diagrams, the score is checked or underlined and the page is kept with medical charts). As those considerations are only speculative, we did not include them into the manuscript.

Changes in text: We revised the whole paragraph in regard to the patient selection process (Lines 138-141).

- Do the authors have information about collapse?

Reply 6: We are very sorry for our lack of understanding, but would you kindly explain what do you mean by information about collapse?

Changes in text: None.

- Figure 1: Maybe consider to add percentages.

Reply 7: This is a very accurate suggestion, thank you very much (we believe that you meant "Figure 2", as this consist of the chart with the numbers).

Changes in text: We revised the figure, accordingly (however, please note that the number of this figure is now 1, as we deleted Figure 1 due to copyright considerations).

- Table 1 and/or 2: Consider to add the median number of cores as mentioned in line 167 (try to avoid interpretation of the results in the result section).

Reply 8: Thank you very much for this valuable suggestion. The Tables 1 and 2 are aimed to present the characteristics of the patients, i.e., variables that may be interpreted as patient- or lesion-related risk factors. Number of cores is a procedure-related factor and thus represents different aspect of the possible cause and effect chain. This is why we decided to present it separately from patient and lesion characteristics. In order not to produce a table for one variable, we presented this data in narrative format. We are aware that this may seem not to fit well, but still appears to be the least confusing way.

Changes in text: None. - I have several comments regarding table 3:

i) Consider to take the painscore as a continuous endpoint within this analysis and not dichotomize in insignificant vs significant pain.

ii) number of predictors (n=6) is a little bit high for the number of events (n=55). And I believe not for every predictor is a good rationale. Consider to exclude PSA and DRE from the model, unless the authors can explain why a higher/lower PSA levels or a suspicious DRE might be associated with higher pain scores. I am curious about the predictive value of the number of cores in the multivariable analysis, could the authors maybe include this variable to the model instead of PSA and DRE? (Consider to analyse the number of cores for example per 5 cores for better interpretability).

iii) please define the reference for categorical variables (e.g. biopsy naïve(=ref) vs. previous negative?).

Reply 9: We appreciate those valuable comments aimed to improve the quality of the table. Ad. i) Initially, we did consider taking the pain score as a continuous endpoint for the whole manuscript. However, as most of the values fell at 1 or 2 (or 2 and 4 in the revised version of the paper – we adopted changes suggested by one of the reviewers who asked to recalculate the numbers according to the new version of the Faces scale), this was non-conclusive. In Table 3 we provide the results of logistic regression and thus using a dichotomous dependent variable is necessary.

ii) Table 3 provides the results of univariable models, this is why we were not limited by the number of predictors. This is not a multivariable model. In the revised version of the Table we replaced the word "models" with "univariable logistic regression" to avoid further confusion. Also, for clarification we explicitly stated in the revised version of Results section that we did not proceed with multivariable modeling. PSA and DRE were analyzed as factors as those are very basic characteristics of patients suspected of prostate cancer, easily available in every man undergoing biopsy, and we felt obliged to include them into the analyses. In regard to the number of cores: as Table 3 was aimed to analyze patient-related factors, number of cores do not fell into this category. The table heading might have been confusing; this is not multivariable modeling. Results of univariable analysis in regard to number of cores are included in the Results section: "on a univariable model, increasing number of cores was negatively associated with SP (OR 0.86, 95% CI: 0.75-0.97)". When working on the manuscript we did develop a multivariable model that included patient age and number of cores. Both of the variables demonstrated significant association with SP (Age: OR 0.93 [0.88-0.98, p = 0.006]; # of cores: OR 0.86 [0.76-0.98, p = 0.026]). However, given the low clinical significance of such model (anticipation of pain level is necessary prior to the biopsy not after the number of cores is known), we decided not to include it into the paper.

iii) We revised the table and added this information in the footer, thank you.

Changes in text: We revised Table 3. Also, please see lines 148-150.

- Table 3: consider to include age per 10 years in the regression analysis for better interpretation of the OR.

Reply 10: Thank you very much for this suggestion. Now the OR looks much clearer.

Changes in text: Please, see the revised Table 3. Also, we revised the Abstract and replaced the old OR with the new value.

- Line 172: regarding the aim of this study, could the authors explain why they included cancer detection rates in the analysis? It seems a bit out of scope. If they believe this is relevant, please introduce this outcome measurement in method section and define csPCa.

Reply 11: Thank you for this comment. We understand that this unexpected introduction of csPC in the Results may seem out of scope. However, we explained it later in the Discussion section. We hypothesized that in patients with an ongoing chronic inflammatory process in the prostate, a condition whose clinical image may rise suspicion of csPC and lead to an unnecessary biopsy, the periprocedural pain would be increased, as prostatitis or chronic pelvic pain syndrome is commonly linked to altered nociception in the pelvis. Adding PC/csPC into the analyzes was aimed to indirectly provide insight into this hypothesis. According to your valuable remark, we implemented proper revisions to the Methods section.

Changes in text: We defined csPC defined in the methods section (Lines 120-121).

- Line 174: Absolute numbers and confidence intervals are missing.

Reply 12: Thank you very much for noticing this. We added the absolute numbers. However, as these measures are describing ratios, confidence intervals are unavailable. The P-value refers to Chi-square comparison. ORs are not calculated, as we aimed only to compare the groups.

Changes in text: We included the absolute numbers (Lines 168-169).

Discussion section:

- Line 181: please define tolerate (in terms of pain in this case) since the procedure can still be invasive in terms of e.g. discomfort (positioning, rectal examination, embarrassment). In line with this maybe the authors could elaborate on other aspects than pain only further in the discussion section. A nice referral regarding future research would be to the recent publication of a study protocol that includes PROMS at different moments in time: DOI: 10.1111/bju.15978 (https://pubmed.ncbi.nlm.nih.gov/36695816/)

Reply 13: Thank you very much for raising this interesting and important issue. Firstly, we revised the “well tolerated” as “well tolerated in terms of pain”. Secondly, now we elaborated on the topic of different aspects of discomfort in our discussion. Thirdly, while we do not feel ourselves competent enough to provide comments in regard to the protocol of the TRANSLATE trial, as this reference fits the scope of our manuscript, we mentioned this interesting trial in the Introduction

section, at the paragraph describing the contemporary evidence in regard to comparison of biopsy techniques.

Changes in text: Revised as described above. Please, see lines 48-49, 174-177, 208-213.

- Line 181: please avoid subjective wording (18% instead of less than one fifth).

Reply 14: We are sorry for this judgmental narration; we revised the sentence accordingly.

Changes in text: Revised accordingly.

- Line 193: Consider to add reference (https://pubmed.ncbi.nlm.nih.gov/36631536/)

DOI:10.1038/s41391-022-00641-3

Reply 15: Thank you very much for this very important and valuable remark. However, this sentence refers to "overall" pain scores, as our aim was to present our results (overall pain) on the background of other similar data. The very interesting study by Hogenhout et al. provides pain data for particular stages of the procedure, however, not the overall pain scores, so this reference does not fit into this sentence. We decided to refer to this study later in the discussion section (see Reply 17).

Changes in text: None (applied later).

- Line 195: I am not able to check the reference, but is there no methodological reason for this high pain score rather than just an outlier (e.g. younger patient population).

Reply 16: Mean age in this patient cohort was 61 which is in fact lower than our median age. However, we believe that this does not explain this high degree of outlying. While this referenced study has been published in a peer-reviewed journal, also, it is widely indexed and highly cited, we considered it appropriate to include the results in our discussion.

Changes in text: None required.

- Line 201: again consider to add reference (https://pubmed.ncbi.nlm.nih.gov/36631536/)

DOI:10.1038/s41391-022-00641-3

Reply 17: Once again, thank you for suggesting this reference. It perfectly fits here.

Changes in text: Added the reference (Line 197).

- Line 240-242: could the authors give an explanation for this?

Reply 18: Thank you for raising our attention at the confusing way this sentence was constructed. We just wanted to explain why we considered being biopsy-naïve as a factor for increased pain. We rephrased this sentence.

Changes in text: We made corrections (Lines 243-245).

- Line 242-243: the same question here, this is just a repetition of the findings without explanation. Besides, I would leave DRE status and PSA level out of the analysis as mentioned previously.

Reply 19: Thank you for this comment. We deleted this sentence, in line with your comment. However, as explained above, we believe that including DRE and PSA is important. Firstly, those are easily accessible patient-related parameters assessed in every patient undergoing biopsy and not analyzing them would lead to much more questions than doing so. Secondly, this was also aimed to indirectly search for an association between non-cancer patients and cancer patients, as we hypothesized that non-cancer patients, i.e., patients more likely to be prostatitis patients, would be experiencing more (or less?) pain.

Changes in text: Deleted the sentence in its current form. Discussed only PV here. Discussed DRE and PSA at the prostatitis/csPC/PC paragraph. Please, see lines 246-275.

- Line 262: Please do not use the word "trend" in this context (p values are not moving). So there was a difference in csPCa detection rate between men with SP and non-SP men, the difference was, however, not significant. Confidence intervals are mandatory for interpretation (see also comment regarding line 174). Furthermore, an extra underlying mechanism of men with SP that were less likely to be diagnosed with csPCa is that less cores were taken in men with severe pain (undersampling theory). Maybe the author could consider to mention this theory since it is in line with their own findings discussed in the previous paragraph. Also in line with their own findings, and therefore maybe worth mentioning, is that the incidence of csPCa among younger men is lower and these younger men are at risk of experiencing severe pain. Apart from all this, I am still unsure about the added value of seeking for an association between csPCa detection and pain. What is the clinical implication/consequence?

Reply 20: Thank you for this valuable comments. Firstly, we deleted the word "trend" and replaced it with just "no significant difference". Secondly, we are unable to provide confidence intervals, as we did not calculate values for comparison (odds ratios), just performed a Chi-square to check whether any difference in ratios was significant. In line with your previous suggestion, we added absolute numbers so that the readers can reproduce the test and have better insight into the size of comparison. Thirdly, thank you very much for the suggestion in regard to the undersampling theory. However, as we did not actually find a significant difference between csPC rates in SP and non-SP patients, we have no grounds to discuss it in regard to our results. We added those considerations in the context of discussion on association between pain and number of cores. Fourthly, thank you for the comment in regard to possible interdependencies between age, csPC, pain and number of cores. This is a very interesting topic of discussion, however, we believe that it fells beyond the scope of our manuscript, as neither did we investigate for an association between age and csPC in our group, nor did we find an association between csPC and pain. Fifthly, you asked for our rationale to investigate for an association between csPC and pain. We did this to indirectly seek

for data supporting a hypothesis of prostatitis being associated with the risk of pain, which is explained in Reply 19.

Changes in text: We have done major revisions to these paragraphs (Lines 266-295).

- Maybe the authors could mention the use of a visual scale (i.e. not numeric) as a strength of their study.

Reply 21: Thank you very much for your appreciation. We are happy that you consider this a strength of our study. Unfortunately, we lack proper references to support the superiority of visual scale in this clinical setting, so we cannot add such a statement to our manuscript.

Changes in text: None.

Conclusion section:

- Maybe the authors could be more specific in their main conclusion regarding "well tolerated". Thus not well tolerated because low levels of pain, but in terms of pain, since toleration encompasses more than just pain as mentioned previously.

Reply 22: Thank you for this important remark. We admit that this was non-specific and judgmental.

Changes in text: Deleted this part of the phrase, left only "associated with low levels of patientreported pain". We made same revisions in multiple sentences in the manuscript.

- Conclusion is quite long including some unnecessary details such as the definition of significant pain and mentioning the limitations of the study (the last sentence that further research is needed seems sufficient). Besides excluding the limitations and the definition of significant pain, the authors could consider to mention younger age as a risk factor for significant pain (and not the opposite; that older age was associated with lower pain scores). Lastly, maybe the authors could consider to include a more concrete clinical implication (like in line 235-238).

Reply 23: Thank you very much for your valuable suggestions aimed to improve our Conclusions. We revised this section accordingly. However, we did not use the word "risk factor" in regard to younger age, as this would inappropriately contrast with the optimistic conclusion of good pain tolerance, moreover, the ORs in the text are calculated for age ≥ 62, not < 62 years old.

Changes in text: Revised the whole Conclusions section.

Reviewer D

Summary:

This study proves that TP biopsy under local anesthesia is well tolerated by the majority of the patients. This technique can indeed help in developing strategies for improving cost-effectiveness of PC diagnosis.

Abstract:

36: TP biopsy is the recommended standard (not becoming recommended standard)

Reply 1: Thank you for this important remark.

Changes in text: Corrected accordingly (Line 3).

52: what is older men? important to mention in the conclusion of the abstract + also mentioning the percentage of SP in older men

Reply 2: By "older" we meant both simply "older", with no threshold, as demonstrated on logistic regression, and "older or equal to 62-year-old", as demonstrated by odds-ratio and Chi-square. The percentage of SP, as well as absolute number, is provided in the Results section of the Abstract. We tried to avoid repeating the numbers, according to editorial policies.

Changes in text: None. Introduction: ok

Materials and methods:

93: can you include PSA density? also in table 2 it would be better to mention PSA density instead of PSA alone, because it reflects better the chance of prostate cancer

Reply 3: Thank you for this valuable suggestion. In the revised version of the manuscript, we have included PSAD into the analyses, finding no statistical difference in regard to PSAD between Center 1 and Center 2 patients (Table 1), no difference between SP and non-SP patients (Table 2) and no association between higher PSAD and SP on logistic regression (Table 3). We also included PSAD in our considerations in the revised Discussion.

Changes in text: Revised all three tables, as specified above. Minor revision of discussion (Line 271).

- is it still possible to include the duration of the procedure? It would be interesting to check the influence of significant pain on the length of the procedure.

Reply 4: This is a very interesting comment and thank you for that. Unfortunately, this is an ambulatory procedure and we do not record procedure duration. Adding this information would be very valuable in terms of possible associations between pain and procedure duration, however, we lack this data.

Changes in text: None.

- was there a difference in reporting significant pain between the two operators?

Reply 5: Thank you very much for this important comment. One of the operators performed biopsies in Center 1, the other performed biopsies in Center 2. Indeed, we noted a significant difference in regard to SP between the two centers: 5/59 (8.5%) in Center 1 vs 50/240 (21%) in Center 2, $p = 0.028$. However, we do not include this data into the manuscript for several reasons. Firstly, we would like to avoid assumptions that one of the operators performs biopsies in a better way in regard to pain or provides more friendly environment, as this would be a hypothesis difficult to justify on a scientifical basis and pointless on the background of the major aim of this goal, i.e., to assess for patient-related risk factors, as in a real-life scenario one cannot predict who will perform the biopsy in a statistical patient. Secondly, we believe that this difference might have been in fact caused by a bias. Please note, that, as demonstrated in Table 1, the patients significantly differed in regard to age between the centers. Patients in Center 2 were significantly older (median age 67 vs 64, $p = 0.01$). In fact, the observed difference in SP rates between the centers (operators) was most probably caused by difference in age of patients. Of course, one may assume that it was the other way, i.e., for some reason patients in Center 2 were older and biopsies in Center 2 were typically less painful, which led to observing a biased association between age and pain. However, association between pain and age is in fact explainable on the basis of medical knowledge, as discussed in the manuscript. Moreover, as shown in Table 1, DRE status, being biopsy-naïve and lesion involving AFS were other factors that demonstrated significant differences between the Centers, but no association with SP was found for these variables. If the hypothesis of one physician performing less painful biopsies than the other one was true, we would be more likely also to observe different rates of positive DRE, being biopsy-naïve and AFS lesions in SP and non-SP patients.

Changes in text: As we feel that the above considerations would add more confusion than true value to the manuscript, we did not include them.

results:

- if you have 18,4% of patients with significant pain it looks still a high number, because 1/6 to 1/5 procedures will be more difficult because the patient is suffering from pain

Reply 6: This is a very interesting and valuable comment, thank you. As stated in the Discussion, our definition of significant pain was arbitrary and mainly aimed at making statistical analyses

doable (dichotomization of pain was necessary for logistic regression and Chi-square testing). There is no evidence-based recommendation what pain to consider significant in regard to this scale and this setting. We could have set the threshold at pain 8/10 (please note, that according to suggestions of one of the reviewers we doubled the pain scores to adjust the results to the updated Wong-Baker Faces scale), making the SP rates lower, i.e., 25/299 (8.4%), which would not change the absolute results, but would change the perception of the results by the readers. However, this low rate of negative events would have made analyses very difficult, and no associations could have been investigated. An important information, stated in the Abstract and in the Results, is that median pain score was 2/10 (IQR: 2-4), which provides most nonjudgmental insight into pain tolerability by our patients. As discussed in the manuscript, other outcomes could be evaluated as a measure of patient tolerability. What was available for us, was the Wong-Baker Faces Scale score. In fact, an interesting area of further studies would be possible associations between pain score and potential negative outcomes (i.e., prolonged procedure, missing targets, complications), which would help in determining what pain is SP in regard to anticipation of negative outcomes.

Changes in text: None.

This can lead to longer procedure time: do you have data of the length of the procedure?

Reply 7: Thank you for this comment. Please see our Reply 4.

Changes in text: None.

This can lead also in more movements of the patients with influence of the cancer detection rate of the biopsy: do you have data of the influence of pain in the diagnosis PC and csPC?

Reply 8: Thank you for this comment. No significant associations were found between pain and PC/csPC diagnosis. However, men reporting SP tended to be diagnosed with csPC less often (31% vs 43%, $p = 0.10$). While the difference was non-significant, a larger study aimed at deeper investigation of this problem would be an interesting idea.

Changes in text: Based on the considerations in regard to Comments 6-8, we discussed the topic of association between pain score and negative events in the Discussion (Lines 301-306).

158: the discrimination of SP for patients younger or older than 62 years old can be helpfull

Reply 9: We are very sorry for our lack of understanding, but would you kindly explain your question? In our study we developed a ROC model and based on this model we found the threshold of 62 years to be optimal. Then, we calculated OR for SP with age 62 or older.

Changes in text: None.

172: This study reports a detection rate of 65.6% PC and 40.8% of csPC. This is not so high in comparison to the study of De Vulder et al. Abdominal Radiology 2022.

Several factors can influence this: (should be mentioned also in the discussion) - how many PIRADS 3, 4 or 5 lesions are included? this influences the CDR of the biopsy

- the local anaesthesia is less in comparison to this study, so maybe the patients where moving more what can influence the CDR of the biopsy

- or was it the influence of a different fusion system?

Probably patients that are having significant pain will move more what influences the CDR of the biopsy (with having less csPC).

Reply 10: Thank you for this interesting comment. In fact, our rates may seem low. High variability of CDR between centers/ populations was described and investigated in the metaanalysis by Mazzone et al. (https://pubmed.ncbi.nlm.nih.gov/33358543/). Please see one of our previous manuscripts: https://pubmed.ncbi.nlm.nih.gov/36614957/, where we present our institutional cancer detection rates for specific PIRADS categories and discuss factors influencing the CDR in our patients. To summarize, most probably those relatively low CDrates are caused by a specific patient population, namely high rate of repeat biopsies. In your comment, you asked for number of patients with particular PIRADS categories. In the revised Tables we have provided the numbers and demonstrated no significant differences between Centers, SP vs non-SP patients and no significant association with SP on logistic regression (PIRADS category 5 was chosen as threshold for dichotomization). In our study we found no significant association between SP and csPC. This topic was discussed by us in Reply 8.

Changes in text: We revised the tables.

Discussion 179: this is not one the largest studies. We present a study to investigate the risk of SP.

Reply 11: Thank you for this remark. We have made proper corrections.

Changes in text: Corrected the text accordingly (Line 173).

200: this study reflects the overall tolerability of this procedure what can be very helpfull in the future.

But a major limitation is that no difference is made between the pain score of injecting the local anaesthesia and performing the biopsies.

. SP during local anaesthesia is acceptable.

. SP during the biopsy is not acceptable because SP leads to more movements of the patients during the procedure and more tension of the pelvic floor muscles. This leads to more problems with a correct fusion between US and MRI. A good fusion is mandatory because this influences the cancer detection rate.

Reply 12: This is a very important comment and thank you for it. We are aware that lack of differentiation between procedure stages for the purpose of pain reporting represent a limitation of our study and we do discuss it in our manuscript. Apart from considerations whether this may have biased the assessment of patient satisfaction, you raised an important topic of significant pain possibly affecting the quality of biopsy. This have been discussed by us in our previous replies. In fact, differentiation between procedure stages might have led to different results, e.g., if we analyzed needle-shot-related pain separately, we might have found that SP related to this stage only was related to lower csPC detection rates. However, it must be mentioned, that other studies cited in our paper demonstrate that most of the pain is perceived during injection of anesthesia, so most probably we would not have recorded many biopsyshot-SP patients.

Changes in text: We have made an additional mention of the above considerations in the paragraph related to possible impact of pain on the quality of biopsy (Lines 291-295).

243: If you hypothesise that the location of the lesion has an influence of the degree of pain it is also important to correlate this with the volume of the prostate. Was the local anaesthesia surrounding enough the volume of the prostate (f.e. in larger volumes)?

Reply 13: Thank you for this comment. In fact, larger prostates could have been a greater challenge in regard to proper injection of anesthesia, however, we found no association between PV and degree of pain (please, see Table 2 and Table 3). As we stated in the Methods section, larger amounts of lidocaine were injected in cases of larger PV.

Changes in text: None.

259: this study tries to give a correlation of cancer detection rate and reporting significant pain. This is not correct. This study reports a detection rate of 65.6% PC and 40.8% of csPC. This is not so high in comparison to the study of De Vulder et al. Abdominal Radiology 2022. Several factors can influence this:

- how many PIRADS 3, 4 or 5 lesions are included? this influences the CDR of the biopsy

- the local anaesthesia is less in comparison to this study, so maybe the patients where moving more what can influence the CDR

- or was it the influence of a different fusion system?

the explanation of the possible inflammatory processes is not relevant for the possible decleration of cancer detection rate and significant pain

Reply 14: Thank you for your valuable and thorough review. This Comment covers with one of the previous comments, please, kindly see the Reply 10.

Changes in text: N/A.

Conclusion ok

Reviewer E

This retrospective study examined patient-reported pain at MRI/US fusion biopsy of the prostate under local anesthesia and any associations with patient characteristics. It is concluded that the proportion of patients who experienced significant pain after biopsy under LA was limited and that a markedly less proportion of older men experienced significant pain.

Although the scale is highly subjective, it is an acceptable outcome because the experience of biopsy is obviously subjective and peculiar to the patient. The cutoff value used for significant pain is highly arbitrary, but the overall concept of the study is clear enough to make a clear point. Namely, that LA is the way to go.

The paper is very well written. Its proper structure and important emphases make it a smooth read.

The introduction gives a nice overview of the current status of prostate biopsy and shows that this is a hot topic, in this way the relevance of the study is well emphasized. The methods and results were clearly written out and illustrated by figures and tables.

In the discussion, important findings are brought out and the study's own limitations are transparently narrated and commented on.

The conclusion is limited to the essentials.

I have no significant comments. Any comments would merely illustrate personal taste and dilute the writers' personal signature.

I find this a very interesting topic where the importance of studies such as this one is rather underestimated.

Reply: Thank you very much for reviewing our manuscript. We truly appreciate your time and effort. We are very content with your opinion. As you did not raise specific questions, we made no changes in the manuscript in regard to your review.

Changes in text: None.

Reviewer F

Szempliński et al. performed a retrospective study of consecutive patients who underwent MRI/US software fusion biopsy of the prostate under LA with lidocaine at two centers between May 2020 and April 2022, and evaluated periprocedural pain using a modified Wong-Baker FACES Pain Rating Scale (0-5). Their study outlines that performing TP MRI/US fusion prostate biopsy under LA is associated with low rates of severe pain.

Feasibility and safety of TP prostate biopsy have been extensively studied in recent years. For example, Marra et al. (J Urol. 2020 Dec;204(6):1209-1215. doi: 10.1097/JU.0000000000001234) informed the results of a series 1,008 men undergoing TP fusion biopsies under local anesthesia and they indicated that prostate biopsy using a trasperineal approach results in moderate pain. They also identified age and severe anxiety as protective and risk factors, respectively, for severe biopsy pain.

Reply 1: Thank you very much for the comprehensive and thorough review of our manuscript. We are aware that our results may not be considered novel in view of multiple other papers also having reported distribution of pain scores in patients undergoing TP biopsy under LA. We refer to several of those articles in our manuscript. We also do admit that not considering the study by Marra et al. in our paper was a mistake. We initially considered a study on patients who underwent free-hand biopsies not well-suitable for our discussion and subsequently we missed that this particular study reported age as protective factor, which falls withing the scope of our study. To our knowledge, now we are the first to confirm this finding. In fact, investigation into possible risk factors of increased pain was one of our main goals (the second being overall analysis of pain during TP biopsy under LA). Thank you very much for raising our attention on this valuable study by Marra et al. We revised our Discussion to include their findings.

Changes in text: Please, see lines 197, 225-226 and 229-230.

One of the main limitations of the present study by Szempliński et al. is the scale used to explore the periprocedural pain.

Reply 2: Thank you for this comment. We are aware of this limitation, as many studies reports that pain perceived by the patients differs in regard to the stage of the procedure. We have discussed this limitation in our manuscript. However, still, we believe that the overall patient feedback may be considered a reflection of their general satisfaction with the anesthesia type.

Changes in text: None.

The present study used the periprostatic block method ("a TP bilateral injection of 0.5% lidocaine was administered with a 22-gauge needle to the presumed localization of neurovascular bundles along the posterolateral periprostatic area"). However, according the results of the APROPOS trial (Perineal nerve block versus periprostatic block for patients undergoing transperineal prostate biopsy (APROPOS): a prospective, multicentre, randomised controlled study. He, Bi-MingWang, Haifeng et al. eClinicalMedicine, Volume 58, 101919), a randomised controlled trial that compares

the perineal nerve block and periprostatic block, the former was superior in pain control for men undergoing a transperineal prostate biopsy.

To sum up, unfortunately, the results from this study are well-known and there is a lack of innovation in this research.

Reply 3: Thank you very much for providing this valuable reference. In the revised version of the manuscript, we discussed the findings of the APROPOS study. Also, we admit that our study was not aimed to investigate a novel technique. Instead, we rather planned to provide results that could be useful in a typical clinical scenario.

Changes in text: Please, see lines 190-192.