**Peer Review File**

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**Round 1**

**Reviewer A**

Comment 1.

*This collection of samples, including blood, CSF, and clinical data (with specific pain outcome questionnaires among others) is of great interest to advance the knowledge of neuroimmune mechanisms in chronic pain patients.*

*I have no problem with the content of the manuscript and possible weaknesses have already been detected and declared by the authors.*

*I only missed that the authors explain how and when they will carry out the analysis of neuroimmune and glia-related biomarkers in the different samples collected.*

Reply to comment 1.

We thank Reviewer A for this assessment. The analysis of neuroimmune and glia-related biomarkers is ongoing. This information has been added at page 6, line 14.

Changes in the text: "The data collection has now ended and biomarker analysis is ongoing."

Comment 2.

*Another question is whether the biobank go on collecting samples indefinitely*

Reply to comment 2.

No, we have ended the collection of samples and present the final cohort in this manuscript. This information has been added at page 6, line 13-14.

Changes in the text: "The data collection has now ended and biomarker analysis is ongoing."

**Reviewer B**

Comment 3

*The manuscript is overall great written and I only have some minor comments as described below.*

*In the method you should include NRS cut-off score for inclusion.*

Reply to comment 3.

We thank Reviewer B for this assessment. We did not use an NRS cut-off score for inclusion in the study. Rather, patients were included if their chronic pain caused substantial restrictions in their functional level.

The high impact pain group is defined in the US National Pain Strategy as "chronic pain with substantial restriction in work, social and self-care activities for at least 6 months." This is outlined on page 8, line 12-13. We believe this definition better illustrates the impact of chronic pain on the affected patients, than the specific NRS-value.

Regarding the Osteoarthritis-group, the indication for alloplastic surgery was pain leading to functional impairment. The indication was based on a general assessment of the patient by the surgeon, rather than a specific NRS cut-of value and again we argue that the functional impairment caused by the pain better illustrates the impact of chronic pain than the NRS-value it self. We have added the indication for surgery on page 8, line 18-19.

Changes in the text: on the indication pain with reduced functional ability.

Comment 4:
*The age range of the participants should ne included in the method section.*

Reply to comment 4: We acknowledge this point and have modified the text to include this information on page 8, line 10.
Changes in the text: "All participants were between 18 to 80 years of age."

Comment 5:
*Were all the samples centrifuged at 2000 G for 10 min, then please clarify.*
Reply to comment 5: All blood samples for plasma and serum storage, were centrifuged at 2000 G for 10 minutes. However, samples for full-blood were not centrifuged according to standard laboratory requirements. This has been clarified by modifying the text on page 9, line 13. All CSF-samples were centrifuged at 2000 G for 10 minutes as outlined in the text on page 10, line 4.
Changes in the text: "All blood samples for serum and plasma, were centrifuged at 2,000 G for 10 minutes."

Comment 6:
*What will happen further on? Are you going to include more samples in the biobank and how will these samples be used?*
Reply to comment 6: We are pleased by the interest in the future for our biobank. As outlined above, no more samples will be included. Laboratory analysis is ongoing. This information has been added at page 6, line 13-14.
Changes in the text: "The data collection has now ended and biomarker analysis is ongoing."

Comment 7:
*Would it be possible to combine figure 1A, 1B and 1C to one chart to visualize the inclusion process?*
Reply to comment 7: We thank reviewer B for this input to improve the overview of the inclusion process and has modified figure 1A, 1B and 1C into one chart.
Changes in the text: We have modified figure 1 and refer to the revised figure.

Comment 8:
*In table 1 statistical differences between groups if significant should be described. For table 2 and 3 statistical differences, if any, between pain groups would be interesting to discuss.*
Reply to comment 8: We agree that describing significant group differences in table 1, 2 and 3 will improve the insight to the data and we have added this to the revised table 1, 2 and 3. We have also added the descriptions of the results on page 17, line 3-7 and line 12-15 and modified the discussion on page 19, line 12-14 and modified the text on page 20, line 7.
Changes in the text:
"Mean age and BMI was significantly higher in both pain groups compared to the pain-free group while physical activity was significantly was significantly lower. Participants in the HI-pain group smoked less and drank less alcohol than the participants in the pain-free group while participants in the OA-group had a slightly higher alcohol intake than participants in the pain-free group. Overall, smoking and alcohol consumption were relatively limited in all three groups."

"NRS scores between the two pain groups did not differ, but participants in the HI-pain group had significantly longer pain duration and widespread pain index and rated themselves worse on both scales for functional impairment (PDI and FIQR), anxiety, depression and insomnia."

"Interestingly, the pain scores in the two groups were similar but the impairment of physical function and psychological health scores, were significantly different in the two groups, indicating that pain disability is not solely determined by pain intensity."

"First, the basic demographic variables, especially age, varied significantly among the three groups"

**Reviewer C**

Comment 9:

*In this manuscript, Blichfeldt-Eckhardt et al report the status and baseline characteristics of participants in an ambitious chronic pain biobank project that has (one understands) now been closed for inclusion. The main novelty and strength of the project is the collection of cerebrospinal fluid (CSF) in a relatively large number of participants, including healthy controls (although blood samples are also collected). This is an ambitious project, and I congratulate the authors for having achieved such a nice biobank for chronic pain research. It is an impressive work. The paper is well-written and contains detailed descriptions of the material and procedures.*

*For obvious reasons, CSF is a very interesting biofluid for neurological biomarker studies. CSF sampling is difficult, and this difficulty is illustrated by the fact that, although they used what is sometimes called "atraumatic" needles, still one participant was treated with an epidural blood patch because of post-dural puncture headache (PDPH) – showing that, although PDPH is not very frequent when using this kind of needles (1), it still happens, and, when many lumbar punctures are made, rare complications will indeed occur sooner or later. Given the suffering associated with chronic pain in general, and the dire need for better treatment options, such studies can nonetheless be given ethical authorization – as in this and other previous CSF projects (2-5). However, it places a huge responsibility on the involved researchers. To state the obvious: It has to be remembered that there is a huge difference in terms of "invasiveness" between a lumbar puncture and taking a blood sample. This fact only underlines the achievement of the present biobanking project.*

Reply to comment 9: We thank reviewer C for thorough appraisal and we agree that security of the participants is of the highest importance in these kinds of studies.

Changes in the text: We have not made corrections in the text, as no corrections have been called for.

Comment 10:

*There is only one concern or discussion point that, in my opinion, deserves to be called "major" in this context. In their description of study limitations, the authors do not mention what I believe to be the most important limitation, which is as follows. The authors do not seem to have stratified the 201 chronic pain patients into different pain groups. If I am wrong about this, the authors should revise the manuscript accordingly, i.e., be clear about it. If I am right, I think it is important to state this a potential limitation – and in that context, they can (if they want) discuss the advantages and disadvantages of hypothesis-generating (explorative) vs. hypothesis-confirming studies.*

*As we do know little about the exact pathophysiological mechanisms underlying chronic pain conditions, explorative studies are very important. Still, I do not think chronic pain should be studied primarily as an indiscriminate whole. Of course, it is possible that chronic pain conditions*

*as a whole will have a "core" of common pathophysiological mechanisms. Moreover, perhaps our present diagnostic categories do not at all do justice to the real mechanisms (c.f., the concept of clinical disease entities being umbrella terms that encompass several "molecular diseases" that share prominent signs and symptoms (6)). However, I think the author should have been able to stratify the 201 patients in some broad and sensible categories, e.g., chronic widespread pain, chronic low back pain, or neuropathic pain. Hence, the possibility to study not only what is common to different pain conditions, but also what differentiates them. But some of this is perhaps possible to do with the help of PROMS (e.g., the CWP?), or perhaps the authors have ethical authorization to retrospectively stratify patients through diagnoses in medical records? Be that as it may, I think the manuscript would be stronger if this issue were somewhat discussed.*
Reply to comment 10: We agree to this insightful comment and have stratified the 201 patients into categories based on pain distribution in the revised table 2.
Changes in the text: We refer to the revised table 2.

Comment 11:
*Title: I would suggest the value-laden word "unique" should be deleted from the title. Better let the reader be the judge of that. Perhaps instead state how many participants you have? Same in the abstract*
Reply to comment 11: We accept the argument from reviewer C and have deleted the term unique from the title.
Changes in the text: The Danish Pain Research Biobank (DANPAIN-Biobank): A collection of blood, cerebrospinal fluid, and clinical data for the study of neuroimmune and glia-related biomarkers of chronic pain.

Comment 12:
*Abstract: "Quantitative tests" is not perhaps the best way to express it. Perhaps "psychophysical" instead?*
Reply to comment 12: We believe that "Quantitative sensory tests" is the generally recognized term for these tests (*Value of quantitative sensory testing in neurological and pain disorders: NeuPSIG consensus. Pain. September 2013, volume 154(9),p1807-1819, https://www.iasp-pain.org/publications/relief-news/article/qst-musculoskeletal-pain/*). We have added the word sensory on page 3, line 17 but if the editor insists, we will use the term proposed by the reviewer.
Changes in the text: "Quantitative sensory tests were performed…"

Comment 13:
Page 5, line 7: *You state that diagnoses rely on subjective reports. I guess you mean that the presence of chronic pain relies on subjective reports.*
Reply to comment 13: We agree with reviewer C and has modified the text at page 5, line 7-8.
Changes in the text: *"Diagnoses of chronic pain syndromes relies on subjective reports of pain in specific bodily areas, in the absence of biomarkers and concrete objective methods…"*

Comment 14:
*Page 6, lines 6-8: I think you should nuance and be more precise here, especially concerning the word "usually". As it now stands, you give the impression that the 4 studies you previously refer to*

*(ref 28-31) have "surrogate normals" and not truly healthy controls – but actually, all four had healthy individuals and not "surrogate normals" as controls.*
Reply to comment 14: We acknowledge that we should nuance this statement and have modified the text at page 6, line 6-8. However, we hold on to the position that the CSF-control subjects in the study by Kadetof et al from 2012 were "surrogate normals" as they were classified as "*Patients with non-inflammatory neurological symptoms (NINS) were used as CSF controls*". We have added more studies to exemplify the described challenges.
Changes in the text: "First, sample sizes are relatively small, limiting the statistical power. Second, control groups sometimes consist of participants with other diseases rather than healthy volunteers, which potentially can limit their value as a reference group.

Comment 15:
*Page 6, lines 8-10: This characterization needs to be referenced and perhaps nuanced.*
Reply to comment 15: We acknowledge that we should nuance this statement, which is in continuation of the above statement and the included references. We have modified the text on page 6, line 8-10 to clarify this.
Changes in the text: "Finally, some of these studies have important variations in obtaining, handling, and storing samples, which may introduce important systematic bias and limitations to the results and conclusions."

Comment 16:
*Page 7, lines 3-10: Were samples of surgery patients collected during the same period as the others (2017-2019)?*
Reply to comment 16: We thank reviewer c for pointing this lacking information out. It has been added to the text on page 7, line 10.
Changes in the text: Collection of blood, CSF, and clinical data on patients scheduled for arthroplasty surgery of the hip or knee due to painful osteoarthritis (OA-group) was performed at the Department of Orthopedic Surgery of the regional Lillebaelt Hospital, Vejle, Denmark from January to June 2018 (Figure 1).

Comment 17:
*The issue of time of sampling (between 7.30 am to 2.30 pm): The authors are transparent about this and provide detailed information in the supplemental. This is very good. But perhaps a few more sentences are warranted in the discussion about this. In what way could that be an advantage (page 18, line 7)? Please explain.*
Reply to comment 17: We thank reviewer C for giving us the opportunity to elaborate on this point. Some neuroimmune biomarkers show circadian fluctuations and some do not. However for many biomarkers, it is not absolutely established whether circadian rhythms are important when comparing results between groups. We hope to bring new light to this, when exploring the biobank samples. We have modified the text on page 20, line 11-16.
Changes in the text: For those biomarkers where circadian rhythms in the CSF is unclarified, it could bring new knowledge to the importance of the timing of sample collection. For biomarkers with circadian fluctuations it could introduce potential bias, but it should be possible to correct for this potential bias statistically, since the material contains samples from all time points during the day in all three groups.

Comment 18:
*Page 16, line 3, and Table 1: The authors only report descriptive data in Table 1. I would suggest that it is important to inform the reader about the possibility of statistically significant differences at baseline (although the authors already do mention this as a limitation).*
Reply to comment 18: We agree that adding information on statistically significant differences at baseline will improve the manuscript as also pointed out by reviewer b. We have added this information in the revised figure 1.
Changes in the text: We refer to the revised figure 1.

Comment 19:
*Page 17, lines 11-12: "... a variety of pain syndromes". See the "major" question discussed above.*

Reply to comment 19: We acknowledge the need to elaborate on the descriptions of different pain syndromes represented among the biobank participants and have included information on pain classification in the revised table 2 as outlined in the answer to comment 10.
Changes in the text: We refer to the revised table 2.

Comment 20:
*Page 17, line 13: There are studies with healthy controls (the authors refer to them elsewhere), albeit small. Given the paucity of CSF studies in the field, the word "usual" is a very strong one to use here.*
Reply to comment 20: We acknowledge this point made by reviewer C and has rephrased the sentence on page 18 (before revision, page 17), line 16.
Changes in the text: "We believe that the inclusion of 70 pain-free volunteers who were included for the biobank alone and not via other contacts to the health care system provides advantages compared with several other CSF biomarker studies."

Comment 21:
*Fig 1B: Typo.*
Reply to comment 21: We thank Reviewer C for pointing this out. Figure1 has been revised according to comment 7 from reviewer B.
Changes in the text: We refer to the revised figure 1.

Comment 22:
*General about Tables: A bit more information in table titles would be nice.*
Reply to comment 22: We are not quite sure which information is specifically warranted but we have aimed at improving the table titles in order to make them more informative.
Changes in the text: Table 2: Pain outcomes of participants in the DANPAIN biobank.
Table 3: Clinical outcomes of participants in the DANPAIN biobank.
Table 4: Postoperative outcomes of participants undergoing hip and knee arthroplasty because of painful osteoarthritis.

Table 5: Quantitative sensory testing outcome in participants with high impact chronic pain and painfree volunteers.
Table 6: CSF analysis of participants in the DANPAIN biobank.

Comment 23:
*Clarify what you exactly mean by "extraction time".*
Reply to comment 23: In supplementary figure 1, extraction time refers to the time from needle insertion until the sample was fully collected (through a 25-27G needle). We have changed the wording to "sample collection time" in supplementary figure 1.
Changes in the text: Sample collection time.

Comment 24:
*Conclusion*
*In my opinion, it is probable that this ambitious project will generate a lot of interesting and important pain biomarker data in the future. There is a dire need for more biomarker studies, but they are still few in the field. For instance, a recent systematic review of proteomics and chronic pain revealed only 27 papers, the authors concluding that the field is still in its infancy (7). Projects such as this one will surely lead the field to a greater measure of maturity – and perhaps, in the future, to better treatments for chronic pain patients. I applaud the authors for their important contribution.*
Reply to comment 24: We are grateful for this appraisal by reviewer C.
Changes in the text: None.

**Reviewer D**

Comment 1:

*The DANPAIN-Biobank study appears to provide potentially valuable data to the pain research world. I appreciate the efforts made by this group. The manuscript would be of value for publication but would benefit from additional edits to provide greater clarity to the study protocol and improve the readability. Considering the stated purpose of the manuscript is to describe the methods of the study, further detail to study methods is warranted. Especially if data will be made available to the greater research community, which was not discussed in the discussion or conclusions? There is a great focus on neuroimmune mechanisms in the introduction yet no mention of assays in the methods? I would recommend an additional review for grammar, punctuation, and capitalization.*

*Abstract*

*After reading the manuscript, it appears this manuscript is focused on the study protocol and brief population descriptions. I would reinforce this in the abstract. As it reads now, I am expecting more data.*

*I would suggest reinforcing the purpose as written in the introduction.*

Reply to comment 1: We thank Reviewer D for this appraisal and have reinforced the abstract by a reinforcing the purpose as written in the introduction on page 3, line 11-12.
Changes in the text: "In this paper, we describe the methods and the study population of the DANPAIN-Biobank."

Comment 2:

*Introduction*

*Page 5, Lines 21-22: a brief description of "further immune functions" would be beneficial to the reader. Particularly considering the focus on immune function in this project.*

Reply to comment 2: We thank reviewer D for the interest in the subject and we have added a brief description to the general immune function of glial cells. Off note, we consider the glia cell's specific impact on pain modulation more important in this project, than the many different general functions they have in the immune response. We have added the information on page 5, line 23.

Changes in the text: (ie. immune regulation of CNS including production of cytokines, chemokines, removing apoptotic cells etc.)

Comment 3:

*Page 6, Lines 3-10: Can you provide a statement to indicate support or no support for the investigation of inflammatory mediators in the CSF from prior literature? Particularly can you relate any of the prior work in humans, specifically the investigation of dorsal horn neurons in humans, doi:10.1093/brain/awab048 , DOI: 10.1139/y2012-014*

Reply to comment 3: We agree with reviewer D that it is important to provide references from prior literature support the rationale for the investigation of inflammatory mediators in the CSF. This has been done in references: 28-34, but references 23-27 are also important for this rationale. According to this and comments from the other reviewers, we have modulated the text on page 6, line 5-11 to state this.

Changes in the text: In humans, several studies have assessed inflammatory mediators in the CSF and demonstrated signs of central inflammation.(28-34) Unfortunately, several limitations make it hard to draw any final conclusions from these studies. First, sample sizes are relatively small, limiting the statistical power. Second, control groups sometimesusually consist of participants with other diseases rather than healthy volunteers, which potentially can limit their value as a reference group. Finally, somemost of these studies have important variations in obtaining, handling, and storing samples, which may introduce important systematic bias and limitations to the results and conclusions.

Comment 4:

*Page 6, Lines 14-15: Can you provide rational to why STROBE guidelines were followed rather than SPIRIT for protocol reporting?*

Reply to comment 4: We thank reviewer D for giving us the possibility to elaborate on our considerations.

We report this manuscript based on the STROBE guidelines for cross sectional studies because we present a cross sectional study, describing an already included cohort with characteristics and initial CSF-results (protein, leucoyte, erythrocyte, glucose-levels). We did not use the SPIRIT guidelines for recording a protocol, because we do not consider this a protocol, as a protocol presents the plan for conducting a study where participants are later included. In this case we concluded the inclusion and initial analyses which is why we find that it cannot be defined as a protocol.

Changes in the text: None, because the reviewer asks for a rationale rather than changes in the text.

Comment 5:

*Materials and Methods*

*Page 7, Lines 6-7: Can you provide further detail to how persistent pain and substantial restrictions of life were assessed? Also, was high impact pain assessed in the OA group?*

Reply to comment 5: The classification of patients as suffering from high-impact pain was based on clinical evaluation. This has been elaborated on page 7, line 7-8. In all patients, pain was assessed using the NRS-scale and functional level was assessed using the Symptom Impact Questionnaire and Pain Disability Index as outlined on page 10, line 18- page 11, line 22.

Changes in the text: The chronic high-impact pain (HI-pain) group was defined as individuals with persistent pain and substantial restriction of life activities lasting 6 months or more and classified as such, based on clinical evaluation.

Comment 6:

*Figure 1:*

*Page 7, Lines 14-15: Can you reinforce if the lumbar puncture was used perioperatively for either/or spinal block or epidural?*

Reply to comment 6: In the OA-group, participants were included from patients who were scheduled for hip or knee arthroplasty in spinal anesthesia. This text has been modified on page 8, line 20 where the inclusion criteria is described, to emphasize this.

Changes in the text: Participants with OA were recruited from patients scheduled for alloplastic surgery in spinal anesthesia at the Department of Orthopedic Surgery, Lillebaelt Hospital,

Comment 7:

*Page 8, Lines 11-13: The US NPS definition of chronic pain is correct but further detail as to how high impact pain was quantified is warranted. Was it only by self-report, clinician assessment, or standardized assessments?*

Reply to comment 7: The classification as patients as suffering from high-impact chronic pain was based on clinical evaluation which has been elaborated by modifying the text on page 7, line 7-8.

Changes in the text: The chronic high-impact pain (HI-pain) group was defined as individuals with persistent pain and substantial restriction of life activities lasting 6 months or more and classified as such, based on clinical evaluation.

Comment 8:

*Page 8, Line 18: Further clarification is warranted to what surgical procedures were enrolled. In previous locations in the manuscript the group is indicated as hip/knee arthroplasty. This line indicates "alloplastic surgery" which may involve procedures which use any graft or synthetic material such as ligament repair and other. Is the population limited to those undergoing hip/knee joint arthroplasty or is this a wider group?*

Reply to comment 8: The participants included in the OA-group were patients scheduled for arthroplasty surgery of the hip or knee in spinal anesthesia. We clarified this by modifying the text at page 8, line 20.

Changes in the text: Participants with OA were recruited from patients scheduled for arthroplasty surgery of the hip or knee alloplastic surgery in spinal anesthesia

Comment 9:

*Page 8, Lines 24-25 & figure 1: Additional exclusion criteria is provided in Figure1C, "surgery in general anesthesia." Can this be explained? Arthroplasty is typically done under general anesthesia, why were these 120 individuals excluded?*

Reply to comment 1: We only included participants who were planned to be operated in spinal anesthesia because lumbar puncture would be performed in these patients whether or not they participated in the study. And thus it was not relevant to include patients who were operated in general anesthesia. In Denmark arthroplasty is typically done in spinal anesthesia, but during the last few years, general anesthesia for this patient group is becoming more frequent for logistic reasons. We clarified the inclusion criteria by modulating the text at page 8, line 20.

Changes in the text: Participants with OA were recruited from patients scheduled for arthroplasty surgery of the hip or knee alloplastic surgery in spinal anesthesia

Comment 10:

*Page 9, Line 10: further description of "plain tubes" is warranted. Were these tubes also centrifuged with similar parameters to EDTA tubes?*

Reply to comment 10: Yes, all blood samples – both in plain tubes and in EDTA-tubes were centrifuged with similar parameters. To clarify this, the text has been modified on page 9, line 15.

Changes in the text: All blood samples for serum and plasma, were centrifuged at 2,000 G for 10 minutes at 4$^{o}$ C.

Comment 11:

*Page 11, Lines 8-9: Further detail to the reference point for pain pattern is needed. Were participants asked relative to the past 7 days, 3 months, 6 months?*

Reply to comment 11: Yes, as outlined on page 11, line 15, pain intensity was assessed as the worst, least and average during the last week as well as pain during physical activity during the last week. This has been clarified by modulating the text on page 11, line 19.

Changes in the text: Pain intensity (worst, least, and average pain) and pain during physical activity during the last week were scored on a numerical rating scale (NRS) ranging from 0-10,

Comment 12:

*Page 10, Line 2: Please provide further detail to "plain tube"*

Reply to comment 12: We do not quite understand the question? We believe that "plain tube" is the commonly used term for blood collection tubes with no coating or active substance such as EDTA, Heparin etc.

Changes in the text: No changes in the text was made.

Comment 13:

*Page 10, Line 4: what kind of sarstedt tube was used?*

Reply to comment 13: We used 0.5 ml polypropylene Sarstedt tubes. The text has been modified on 10, line 7.

Changes in the text: Here they were centrifuged at 2,000 G for 10 minutes at 4o C, aliquoted on ice blocks into 0.5 ml polypropylene Sarstedt tubes

Comment 14:

*Page 10, Line 6 / Supp Table1: Why is there such a discrepancy in the OA group for time from sampling to freezing of plasma?*

Reply to comment 14: We expect reviewer D to be referring to the discrepancy between time from sampling to freezing of plasma samples in the OA-group compared to the two other groups and we do not have a precise explanation for this. The sample collection in the OA-group was done on a different location than for the other two groups (as outlined in the "setting-section" page 7, line 2-

11). It seems that the overall process for blood sample collection, transport to the laboratory and sample handling at the laboratory, differed between the two institutions.

Changes in the text: No changes in the text were made because no changes in the text were called for.

Comment 15:

*Page 10, Clinical data and pain outcomes section: The first paragraph highlights multiple areas of assessment which are not described later in the text. This includes smoking and alcohol habits, physical activity, fatigue, cognition, headache, abdominal pain. Considering this is a description of the study protocol further detail as to how these constructs were measured is needed. Perhaps the authors can organize this section separated by construct to improve the readability of the manuscript.*

Reply to comment 15: We thank reviewer D for the possibility to elaborate on our method and have elaborated on the constructs on page 10, line 14-19.

Changes in the text: Furthermore, participants were asked to estimate their general physical activity into either "primarily sedentary purposes, physical activity at least 4 hours per week, active sports/heavy work or competitive sports and to rate the experience of general symptoms of fatigue, waking unrefreshed, reduced cognition into: no problems, mild problems, moderate problems or severe problems. Finally participants were asked whether they had experienced, headache or abdominal pain during the last 6 months.

Comment 16:

*Page 11, Lines 9-10: what kind of body chart was used?*

Reply to comment 16: An online body chart was used. We clarified this by modifying the text at page 11, line 17.

Changes in the text: Pain distribution during the previous week was reported on the online body chart.

Comment 17:

*Page 11: going back to a previous comment, was the PDI or FIQR used to assess high-impact pain? Specifically the disability/functional limitation component?*

Reply to comment 17: We assessed pain disability and functional impairment using the PDI (pain disability index) and the SIQR (symptom impact questionnaire) but not the FIQR (revised fibromyalgia impact questionnaire). The term SIQR is used in the text, in table 3, we corrected the typo FIQR into SIQR.

Changes in the text: In table 3, we corrected the typo FIQR into SIQR.

Comment 18:

*Page 11, Postop outcome questionnaires section: Were these measures evaluated at baseline as well? According to Table 4, preop measures were taken as well. I would consider clarifying this in the text.*

Reply to comment 18: Yes, baseline scores were also available and we agree that this information should be included which has been done on page 12, line 5.

Changes in the text: In the OA group, postoperative outcome data at 3 and 12 months as well as baseline scores, were available

Comment 19:

*Page 12, Lines 1-3: Was general health only measured in the OA group? If so, why? This seems to be a pertinent assessment*

Reply to comment 19: The EQVAS5D was part of the standard follow-up scores on patients for hip or knee arthroplasty at the Department of orthopedic surgery at Lillebaelt Hospital, Vejle which is why it was available on the OA-patients.

Changes in the text: No changes are made as no changes are called for.

Comment 20:

*Page 12, Lines 6-7: were the QST assessors trained and were any processes instilled to prevent drift among the assessors?*

Reply to comment 20: The assessors had extensive experience and supervised training in the QST-protocol which have been used on several hundred patients in other studies (https://pubmed.ncbi.nlm.nih.gov/26963852/;https://pubmed.ncbi.nlm.nih.gov/36728470/). This has been clarified by modifying the text at page 12, line 17.

Changes in the text: Two operators with extensive experience and supervised training in the QST-protocol (16,57).

Comment 21:

*Page 12, Lines 10-11: the abbreviation for HPT is missing. How was "most painful area" determined in the healthy, pain-free group? What instruction was provided to participants to differentiate heat detection vs heat pain?*

Reply to comment 21: We thank reviewer D for pointing out the missing information. The abbreviation for HPT is added at page 12, line 22. The information that the same area on the lower

back was used on participants in the pain-free group instead of the most painful area and instructions for sensitivity to heat stimulus is added at page 12, line 23 – page 13, line 1.

Changes in the text: Heat detection threshold (HDT) and heat pain threshold (HPT) were assessed over the most painful area and the thenar eminence of the left hand using a MSA Thermotester (Somedic AB, Hörby, Sweden).

In pain-free participants the same area on the lower back was used as substitute of the most painful area. HDT were defined as the first subtle change of temperature and HPT as when the heat from the thermode was experienced as pain.

Comment 22:

*Page 12, Lines 17-18: How was most painful area determined in the healthy, pain-free group?*

Reply to comment 22: This has been outlined above.

Changes in the text: As outlined above.

Comment 23:

*Page 12, Lines 17-23: How was TSP measured? When were pain ratings provided?*

Reply to comment 23: We agree that the information given here is incomplete and has been clarified by modulating the text on page 13, line 8-14.

Changes in the text: To assess temporal summation of pain (TSP), pain intensity ratings after a single pinprick was compared to ratings after a train of 10 stimuli of the same force (repeated at a 1/s rate and given within an area of 1cm2) over the most painful area and the thenar eminence of the left hand. Pain intensity ratings were provided on 0-10 numerical rating scale, where 0 was "no pain" and 10 was "worst imaginable pain". TSP was calculated as the ratio between NRS-scores after the train of 10 stimuli and the single pinprick, with positive values indicating an increase in NRS-scores during the repeated stimulation.

Comment 24:

*Page 13, Lines 2-5: was the pressure increased simultaneously on cuffs on both legs? If yes, has this protocol been validated to show participants experience equal amount of pain in both legs to the same pressure stimulus?*

Reply to comment 24: No, the pressure was first increased on left leg, then the right. This has been elaborated on page 13, line 21.

Changes in the text: The cuff pressure was increased by the computer at a rate of 1 kPa/s, and the maximal pressure was 96.5 kPa at one leg at at time (left first, followed by the right leg).

Comment 25:

*Page 12 & 13: It would be beneficial and easier to read QST sections when equations are provided to indicate how each respective measure was calculated.*

Reply to comment 25: We acknowledge that and have added information on page 13, line 12-14.

Changes in the text: TSP was calculated as the ratio between NRS-scores after the train of 10 stimuli and the single pinprick, with positive values indicating an increase in NRS-scores during the repeated stimulation.

Comment 26:

*Methods section: There is not mention at all regarding any immune assays while this is supposed to be a focus. I would recommend including laboratory methods for these processes.*

Reply to comment 26: We are happy to note the interest from reviewer D in the future studies for the biobank. The biobank was collected to be a research reservoir for continuous research in neuroimmune biomarkers in chronic pain. When these laboratory studies have been finished and published, the methods will also be presented. We regret that it is not possible to provide information on immune assays as these are not part of this study.

Changes in the text: No changes in the text.

Comment 27:

*Page 14, Stats section: Was the study powered for the OA group or were only logistic reasons considered for recruitment?*

Reply to comment 27: The plan for inclusion in the OA group was made on the basis of logistic reasons as outlined on page 15, line 12-13 (*For logistic reasons, we planned to include 100 participants in the OA-group*). As this information is already presented in the manuscript, no further changes has been made.

Changes in the text: No changes in the text.

Comment 28:

*Results*

*Page 15, Line 2: The # of individuals consented would be relevant.*

Reply to comment 28: The number of individuals who consented to participate in the study, is given in figure 1, which has been revised.

Changes in the text: Se the revised figure 1.

Comment 29:

*Page 16, Line 10: Once again the general use of the term "functional impairment" should be detailed by the exact measure used for this construct. There is confusion since the study included multiple measures which somewhat capture functional impairment, including the PDI, FIQR, Oxford scale, UCLA scale. I assume this is in reference to FIQR since it is labeled as functional impairment in Table 3?*

Reply to comment 29: We acknowledge that this information should be specified and has changed the text on page 17, line 12-15.

Changes in the text: NRS scores between the two pain groups did not differ, but participants in the HI-pain group had significantly longer pain duration and widespread pain index and rated themselves worse on both scales for functional impairment (PDI and FIQR), anxiety, depression and insomnia.

Comment 30:

*Page 16, Lines 18-19: Thank you for noting the discrepancy in time to freezer for plasma samples. Perhaps further detail in the methods would explain this? Was the sample not taken to the lab until lumbar puncture was completed?*

Reply to comment 30: We agree that there was discrepancy in time to freeze the plasma samples. As outlined in the answer to comment 4, the samples for the OA-group was taken on a different location (Lillebaelt Hospital) and that the overall process for storing plasma was not as fast here. We cannot provide further explanations.

Changes in the text: No changes in the text.

Comment 31:

*The addition of data completion rates among all assessments would be of value and would add to the statements made regarding the vigor of the study.*

Reply to comment 31: We have outlined completion rates in the revised figure 1.

Changes in the text: See the revised figure 1.

Comment 32:

*All Tables: I appreciate the additional text that indicates what a higher/lower score indicates. I would suggest editing these tables (Table 3, 4) to improve readability.*

Reply to comment 32: Table 3 and 4 has been edited to improve readability.

Changes in the text: See revised table 3 and 4.

Comment 33:

*Discussion*

*To reinforce the comment made in materials/methods, the discussion starts off indicating the purpose to detect neuroimmune and glia-related biomarkers of chronic pain but no methods are indicated in the methods.*

Reply to comment 33: That is true. In this study, we describe the methods and the study population of the DANPAIN-Biobank as outlined in the purpose and discussed in comment 1. We cannot provide details on laboratory methods for neuroimmune biomarkers as they were not part of this study.

Changes in the text: No changes in the text.

Comment 34:

*"Extensive clinical data" is used, further detail of this is needed in the methods as only few examples of clinical data are explained in the materials/methods.*

Reply to comment 1: We believe that the questionnaire data, including specifications of pain outcomes together with the quantitative sensory tests comprise a quite comprehensive collection of clinical data which will potentially support the interpretation of future laboratory tests on sample data quite well. This is off cause our own assessment which can reasonably be argued. We believe however that this assessment is supported by the other reviewers.

Changes in the text: No changes in the text.

Comment 35:

*Page 17, Line 12-15: this sentence is somewhat difficult to understand as written.*

Reply to comment 35: We accept this point and have modified the sentence on page 18, line17-18.

Changes in the text: We believe that the inclusion of 70 pain-free volunteers who were included for the biobank alone and not via other contacts to the health care system provides advantages compared with several other usual practice for CSF biomarker studies testing potential biomarkers in a control group that resembles the background population.

Comment 36:

*Page 17, Lines 16-17: The use of the term "comprehensive data" is not justified as to how the methods/materials are currently written. Greater detail is needed.*

Reply to comment 36: As discussed in comment 34, we believe that the questionnaire data, including specifications of pain outcomes together with the quantitative sensory tests comprise a quite comprehensive collection of clinical data which will potentially support the interpretation of future laboratory tests on sample data quite well. This is off cause our own assessment which can reasonably be argued. We believe however that this assessment is supported by the other reviewers.

Changes in the text: No changes in the text.

Comment 37:

*Page 17, Lines 19-21: further detail regarding the rigor of immune assays is warranted considering the focus of the study.*

Reply to comment 37: Immune assays is not part of this study as previously discussed in comment 33. Therefore we can unfortunately not give details on them.

Changes in the text: No changes in the text.

**Round 2**

1.Concerning Comment 10: The authors have stratified the 201 patients in 3 categories: widespread pain, back pain, and other. 71% of patients have widespread pain. Hence, the biobank is mainly about 2 patient categories: CWP and osteoarthritis pain. Perhaps it would be good, as a minor revision, to make that clear also in the abstract (ie, help the reader at the outset to "visualize" what kind of pain patients we are talking about).
Reply: We acknowledge this good point and have added this information to the abstract.

2. In answer to comment 18, you write about a revised Fig 1; I guess it is a typo, because you have revised Table 1 as I suggested.
Reply: Yes, this is a typo.