PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Efficacy of low-level laser therapy on pain and disability in knee osteoarthritis: A systematic review and meta-analysis of randomized placebo-controlled trials
AUTHORS	Stausholm, Martin; Naterstad, Ingvill; Joensen, Jon; Lopes-Martins, Rodrigo; Sæbø, Humaira; Lund, Hans; Fersum, Kjartan; Bjordal, Jan

VERSION 1 – REVIEW

REVIEWER	Iain Rankin NHS Scotland, UK
REVIEW RETURNED	16-May-2019

GENERAL COMMENTS	The systematic review and meta-analysis have been well performed. The paper would benefit from authors providing further detail in the introduction as to the reported mechanism of action of LLLT to produce its anti-inflammatory effect. Trials assessing LLLT in conjunction with another therapy e.g. exercise, should be meta-analysed separately from LLLT alone. There is a large amount of heterogeneity in the treatment protocols of the trials. The authors should address this further in the discussion and include the recommended protocol. Page 10, line 8: the conclusion appears too strong given the outcomes reported by the meta-analysis. The minimum clinical important difference (MCID) reported of 19.9mm is greater than most of the outcomes reported in the meta-analysis (page 8 line 23 – 42: 14.23 mm, 15.92 mm, 18.71 mm, 6.34 mm). Two outcomes are above the authors reported MCID: page 8 line 29: 23.23 mm - this value is only just above the MCID and has wide confidence intervals of 10.60 – 35.86 mm; further subgroup analysis of follow-up for 2-4 weeks post-therapy outcome measure shows a greater improvement (page 22 line 31: 31.87 mm) however only 1 of these 6 trials had low risk of bias in all domains. Page 19: only 5 of the 22 trials have no risk of bias in all domains; 8 trials have high risk of bias. Page 23: the funnel plot (pain) shows publication bias This is a well conducted meta-analysis, but the conclusions provided by the authors appear inappropriate given the minimal gain in MCID seen in trials which are mainly not free from risk of

	bias. The benefits are likely to be overestimates of effect given the small studies and significant risk of bias. The quality of the evidence appears to be low and a formal GRADE profiling is required. LLLT trials frequently report low- or very low-quality evidence, contributing to the lack of recommendation from EULAR and OARSI (Page 2 lines 57 and 58). If this is the case, the conclusions must be interpreted with caution. Please see https://community.cochrane.org/help/tools-and-software/gradepro-gdt for further information.

REVIEWER	Ricardo Segurado University College Dublin, Ireland
REVIEW RETURNED	17-May-2019

GENERAL COMMENTS	Well done on a thoroughly well planned and described review. I have performed a statistical review and I have no concerns on your methodology. Some minor edits follow which can be amended before publication. 1) Results section (page 4, line 48): 2735 publications identified, the flowchart (figure 1) states 2733 2) Results section (page 8, line 60): p <= 0.007 doesn't appear in the supplementary materials. Perhaps <0.0001 (for concomitant exercise therapy) or =0.01 (for sole therapy)?

VERSION 1 – AUTHOR RESPONSE

REVIWER 1 Dr. Rakin

Comment by Dr. Rakin: “The paper would benefit from authors providing further detail in the introduction as to the reported mechanism of action of LLLT to produce its anti-inflammatory effect.”.

Our response: The working mechanisms of action regarding the anti-inflammatory effects of LLLT are not yet well understood. We believe that the in vivo findings of Wang et al. (2014) and Pallotta et al. (2012) and are useful and mention them in the introduction of the revised manuscript. Wang et al. found that the effects of LLLT progresses over time, in line with our clinical pain and disability results, and Pallotta et al. found that LLLT reduced inflammation even though COX levels increased. The following text has been added:
“LLLT has been applied to rabbits with KOA three times per week for eight weeks in a placebo-controlled experiment by Wang et al. At the end of treatment week six, they found that LLLT had significantly reduced pain and synovitis and the production of interleukin-1β, inducible nitric oxide synthase and MP-3 and slowed down loss of Metallopeptidase Inhibitor 1. Two weeks later, LLLT had significantly reduced MP-1 and MP-13 and slowed down loss of collagen II, aggrecan and transforming growth factor beta, and the previous changes were sustained (Wang et al. 2014). These findings indicate that the effects of LLLT increase over time.
Pallotta et al. conducted a study on LLLT in rats with acute knee inflammation demonstrating that even though LLLT (810 nm) significantly enhanced cyclooxygenase (COX-1 and -2) expression it significantly reduced several other inflammatory makers, i.e., leukocyte infiltration, myeloperoxidase, interleukin-1 and -6 and especially prostaglandin E2. Pallotta et al. hypothesised that the increase in COX levels by LLLT was involved in a production of inflammatory mediators related to the resolution of the inflammatory process (Pallotta et al. 2012).”

Comment by Dr. Rakin: “Trials assessing LLLT in conjunction with another therapy e.g. exercise, should be meta-analysed separately from LLLT alone.”.

Our response: We have conducted the requested post hoc analyses on exercise vs no exercise therapy as co-intervention. They demonstrated that LLLT was significantly superior to the placebo-control both with exercise therapy ($P = 0.0009$ for pain and $P < 0.0001$ for disability) and without exercise therapy ($P = 0.01$ for pain and $P = 0.008$ for disability) as co-intervention. We have provided this information in the revised manuscript. We have added the related forest plots to the supplementary material since 1) we did not plan to subgroup trials by co-interventions, 2) the post hoc analysis could not explain the statistical heterogeneity and 3) the benefits of LLLT with and without exercise therapy appeared to be exactly the same. The difference in pain results was only 2 mm on VAS and the difference in disability results was 0.01 SMD (supplementary material figure 16-17).

Comment by Dr. Rakin: “There is a large amount of heterogeneity in the treatment protocols of the trials. The authors should address this further in the discussion and include the recommended protocol.”.

Our response: We have addressed this further. We find it plausible that some of the statistical heterogeneity of the overall analyses is associated with the dose subgroup criteria (wavelength specific laser doses per treatment spot) since the mean levels of statistical heterogeneity of the subgroup analyses were consistently lower than the overall levels (figure 2-5). We were unable to identify other differences in the LLLT protocols that impacted the results.

Comment by Dr. Rakin: “Page 10, line 8: the conclusion appears too strong given the outcomes reported by the meta-analysis. The minimum clinical important difference (MCID) reported of 19.9 mm is greater than most of the outcomes reported in the meta-analysis (page 8 line 23 – 42: 14.23 mm, 15.92 mm, 18.71 mm, 6.34 mm). Two outcomes are above the authors reported MCID: page 8 line 29: 23.23 mm - this value is only just above the MCID and has wide confidence intervals of 10.60 – 35.86 mm; further subgroup analysis of follow-up for 2-4 weeks post-therapy outcome measure shows a greater improvement (page 22 line 31: 31.87 mm) however only 1 of these 6 trials had low risk of bias in all domains.”.

Our response: Dr. Rakin argue that the conclusion is too strong by referring to results of the non-recommended LLLT doses (6.34 mm VAS) and overall results which are a mix of non-recommended and recommended LLLT doses (14.23 mm VAS, 15.92 mm VAS) (figure 2-3). However, our conclusion is based on the results of the recommended LLLT doses. The dose subgroup criteria were pre-specified and the recommended LLLT doses proved statistically significantly superior to the non-recommended doses (figure 2-3).
The pain reduction from the recommended LLLT doses was significantly superior to placebo even at follow-ups 12 weeks after the end of therapy, and the difference was greater than 20 mm VAS from the final 4-8 weeks of therapy through follow-ups 6-8 weeks after the end of therapy. Interestingly, the pain reduction from the recommended LLLT doses peaked at follow-ups 2-4 weeks after the end of therapy (31.87 mm VAS highly significantly beyond placebo) (figure 7, supplementary figure 1).

The absolute Minimally Clinically Important Improvement (MCII) of pain depends on baseline pain intensity. It was estimated to 19.9 mm VAS by Tubach et al. in 2005. Tubach et al. later estimated the MCII to 17 on a 0-100 scale (Tubach et al. 2012), and most recently, the MCII was estimated to only 9 units on a 0-100 scale by Bellamy and colleagues (Tubach included) (Bellamy et al. 2015).

Dr. Rakin mentioned that the confidence interval for the estimate of effect 2-12 weeks post-therapy is wide. We are not worried about the width of this confidence interval since it did not cross the MCII reported by Bellamy et al. (2015) (figure 3).
Dr. Rakin underpinned that the "follow-up for 2-4 weeks post-therapy outcome measure shows a greater improvement (page 22 line 31: 31.87 mm) however only 1 of these 6 trials had low risk of bias in all domains.". But the trial with low risk of bias in all domains contributed by far with the most LLLT positive results (58 mm VAS beyond placebo) (Nambi et al. 2016).

Comment by Dr. Rakin: "Page 19: only 5 of the 22 trials have no risk of bias in all domains; 8 trials have high risk of bias.".

Our response: Only 8 of the 132 risk of bias scores were 'high risk'. 7 of the high risk of bias scores were due to inadequate therapist blinding, but the 7 trials with inadequate therapist blinding showed less positive LLLT results than the trials with adequate therapist blinding regarding both pain and disability (supplementary figure 10 and 14). Furthermore, the risk of bias subgroup analyses demonstrated that there was no statistically significant interaction between the effect estimates and any of the risk of bias domains scored, and neither could risk of bias explain the statistical heterogeneity (supplementary figure 8-15). Moreover, in the risk of bias chart the trials were ranked by pain effect estimates and there was no asymmetry in the distribution of risk of bias scores beyond chance (figure 6). Therefore, it is our interpretation that the risk of bias was insignificant.

Comment by Dr. Rakin: "Page 23: the funnel plot (pain) shows publication bias.".

Our response: It is still our interpretation that there is no asymmetry in the funnel plot of pain results beyond chance, i.e. publication bias is unlikely. We also checked for small study bias by reducing the statistical weight of the smallest studies through a change from random to fixed effects models and this led to similar mean effect estimates (from 13.22 to 14.14 mm VAS), indicating that small study bias was absent/insignificant, according to the Cochrane Collaboration (supplementary figure 4-5) (Cochrane Handbook chapter 10.4.4.1).

Comment by Dr. Rakin: "This is a well conducted meta-analysis, but the conclusions provided by the authors appear inappropriate given the minimal gain in MCID seen in trials which are mainly not free from risk of bias. The benefits are likely to be overestimates of effect given the small studies and significant risk of bias. The quality of the evidence appears to be low and a formal GRADE profiling is required. LLLT trials frequently report low- or very low-quality evidence, contributing to the lack of recommendation from EULAR and OARSI (Page 2 lines 57 and 58). If this is the case, the conclusions must be interpreted with caution. Please see https://community.cochrane.org/help/tools-and-software/gradepro-gdt for further information.".

Our response: Dr. Rakin summarizes that our conclusion "… appear inappropriate given the minimal gain in MCID seen in trials which are mainly not free from risk of bias.".
Dr. Rakin previously stated that the "The minimum clinical important difference (MCID) reported of 19.9 mm is greater than most of the outcomes reported in the meta-analysis (page 8 line 23 – 42: 14.23 mm, 15.92 mm, 18.71 mm, 6.34 mm)." and we have pointed out that the effect estimates Dr. Rakin mainly are referring to are results of the non-recommended LLLT doses and overall results that are a mix of results of non-recommended and recommended LLLT doses (figure 2-3). As mentioned, our conclusion is based on the recommended LLLT doses as the dose subgroup criteria were pre-specified and the recommended LLLT doses proved statistically significantly superior to the non-recommended doses (figure 2-3). The pain reduction by the recommended LLLT doses had exceeded

placebo by more than 19.9 mm VAS by therapy week 4-8 and it remained above this level through follow-ups 6-8 weeks after the end of therapy (figure 7, supplementary figure 1).

We respectfully disagree with Dr. Rakin that “The benefits are likely to be overestimates of effect given the small studies and significant risk of bias.”. As we explained earlier, our analyses consistently indicated that unclear and high risk of bias was not associated with more positive LLLT results (figure 6, supplementary figure 8-15). Neither did other investigators of reviews on this topic conclude that risk of bias was an issue (Huang et al. 2015, Rayegani et al. 2017). Furthermore, the results of the smallest studies in our review were similar to those of the largest studies (supplementary figure 4-7). In our opinion it would be more inappropriate to neglect these findings and change our conclusion to contradict the data.

Dr. Rakin also stated that and that “LLLT trials frequently report low- or very low-quality evidence, contributing to the lack of recommendation from EULAR and OARSI” and “The quality of the evidence appears to be low and a formal GRADE profiling is required.”. We read the clinical guidelines and found no support for these claims (Greenen et al. 2018, Collins et al. 2018, McAlindon et al. 2014, Fernandes et al. 2013) and would like to point out that EULAR has recently rated the quality of evidence for LLLT in knee osteoarthritis ‘moderate’ (Greenen et al. 2018). Moreover, the PEDro team at the University of Sydney has independently checked the quality of 25 RCTs with LLLT in the knee region and found that two thirds (17 RCTs or 68%) satisfied 60% or more of the 10 PEDro methodological quality criteria.
The suggestion of using GRADE in the manuscript is in our opinion inappropriate, as this tool is not for reviews, but designed to be “a systematic approach for making clinical practice recommendations” (https://bestpractice.bmj.com/info/us/toolkit/learn-ebm/what-is-grade/).

REVIEWER 2

Comment by Dr. Segurado: “… Some minor edits follow which can be amended before publication.

1) Results section (page 4, line 48): 2735 publications identified, the flowchart (figure 1) states 2733”.
Our response: We have revised the flow-chart so that it displays the total number of records identified (2735) as recommended by Dr. Seguardo.

Comment by Dr. Segurado: 2) Results section (page 8, line 60): p <= 0.007 doesn't appear in the supplementary materials. Perhaps <0.0001 (for concomitant exercise therapy) or =0.01 (for sole therapy)?“

Our response: We have updated the P-values for the analysis of exercise vs no exercise therapy:
“Post hoc analyses demonstrated that LLLT was significantly superior to the placebo both with exercise therapy (P = 0.0009 for pain and P < 0.0001 for disability) and without exercise therapy (P = 0.01 for pain and P = 0.008 for disability) as co-intervention (supplementary material).”.