Supplementary material:

The effect of a continuous perineural levobupivacaine infusion on pain after major lower limb amputation: a randomised double-blind placebo-controlled trial

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Recruitment numbers

The data presented are from patients recruited between October 2007 and October 2013. Recruitment slowed during 2012 because the number of patients undergoing major lower limb amputation decreased. We also noted that the overall incidence of late phantom pain appeared to be lower than in our original pilot study. We submitted our blinded data to the independent Data Monitoring Committee in December 2012, who confirmed that the overall incidence of phantom limb pain was lower than anticipated and revised the original power calculation to include 132 patients with evaluable 6-month follow-up data. For these reasons, we submitted a substantial protocol amendment to the LREC and study sponsors to extend recruitment to other centres. However, despite inviting a number of UK hospitals to take part, none were willing to do so because they did not have the resources to commit to the number of follow-up visits required for 6 months after surgery. Recruitment was stopped in October 2013, shortly before the research nurse, who had done most of the recruitment, data collection and follow-up, left our team as her grant funding had expired.

Data handling and statistical analysis

All data were analysed on a per-protocol basis. We calculated the improvement or change of the outcome measure at a given time point by subtracting the score from the corresponding baseline score i.e. change = baseline score – outcome score. Baseline VAS scores at rest and on movement were measured at 6h prior to the operation.

Between one and 96 hours after surgery, patients continued oral morphine perioperatively while some patients received both patient-controlled analgesia device (PCA) and oral morphine. Therefore, we calculated the postoperative morphine usage as total bioequivalent morphine consumption by adding the i.v. PCA morphine use to the daily dose of oral morphine (using a conversion factor of 0.33 so that 10mg oral morphine was classed as 3mg bioequivalent) to give a combined bioequivalent dose.

Daily morphine consumptions (analgesic use) between 24h post-operation to 180d were categorised as no (0), low (1-40 mg), medium (41-100 mg) and high (>100 mg) depending on the actual morphine intake between the specified time and 24h before that time point.

All categorical variables are presented as frequencies (%), and numerical variables summarised as mean (standard deviation) or median (interquartile range) as appropriate.

As a preliminary analysis, we assessed the phantom limb pain and residual limb pain scores between the two groups at each postoperative time point. Since the median scores are zero in most cases, we did not conduct any formal statistical test to compare between groups

We fitted a logistic regression model to evaluate the primary outcome measures, the presence of moderate or severe PLP scores (PLP score ≥ 40) at rest (PLP-R) and on movement (PLP-M) at 6 months, assuming the binomial distribution of the data with a logit link function. The model was adjusted for the baseline score and daily morphine consumption at 6 months regardless of the statistical significance of these predictors. We examined other predictors, such as age, sex, presence of diabetes, types of operation, and preoperative questionnaire data; however, there was no evidence that these predictors were statistically significant ($p>0.05$). All the available data were used to estimate the effect size, and any missing data were assumed to be missing at random.

We also conducted several sensitivity analyses on the primary outcome data on PLP-R and PLP-M scores at 6 months. These include: (a) a general linear model of log-transformed PLP scores at rest

and movement at six months; (b) a general linear model on the improvement in PLP scores at six months. All these models were adjusted for the baseline score and daily morphine consumption at 6 months.

The secondary outcomes included: improvement of (a) phantom limb pain scores at rest and movement at postoperative time points of 24 hours, 48 hours, 72 hours, 96 hours, one week, six weeks, three months, and six months, (b) acute residual limb pain scores at rest and on movement at postoperative time points of 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours and 48 hours, (c) chronic residual limb pain scores at rest and on movement at postoperative time points of 24 hours, 48 hours, 72 hours, 96 hours, one week, six weeks, three months and six months, (d) the presence of non-painful phantom limb sensations and (e) a moderate or higher PSI at 24 hours, 48 hours, 72 hours, 96 hours, one week, six weeks, three months and six months.

We analysed the outcome data on improvement of phantom limb pain, acute and chronic residual limb pain scores at rest and movement for all time points (24h to 6 months) by fitting a separate linear model for each outcome measure. The model included the treatment group and adjusted for the corresponding baseline score (as a deviation from the overall population mean) and relevant data on analgesic use by the patient, i.e. daily morphine consumption for PLP and chronic SP, and morphine bioequivalent dose for acute SP. The model considered time as a categorical variable. We explored the model for other predictors, such as age, sex, presence of diabetes, types of operation, and preoperative questionnaire data (log-transformed), and retained statistically significant ($p<0.05$) predictors in the final model. We evaluated all models using generalised least squares and incorporated autoregressive continuous time process of first-order to account for the correlation between measurements within the same patient, and a constant variance function to account for the heterogeneity of variance of the outcome variable at each time point (i.e. estimating and adjusting for the variance at each time point). All estimates of variances were obtained using the restricted maximum likelihood (REML) method. Details of the modelling framework under

generalised least squares framework are presented by Pinheiro and Bates (2000).[1] For our missing data analysis strategy, we adopted list-wise deletion. No imputation was performed for missing data.

We analysed the data on the presence of phantom limb sensation (PLS) and moderate or greater phantom sensation intensity (PSI) at all time points using a separate mixed-effects logistic regression model. The models were adjusted for treatment, baseline score, daily morphine consumption, and linear and quadratic terms for time points as fixed effects and incorporated patient as a random effect. The estimates of variances were obtained using the REML method.

We also modelled the cumulative bioequivalent morphine consumption at 96h using a general linear model and adjusted for baseline score and other predictors as described above. A comparison of anxiety and depression scores at baseline and 6 months postoperative time point was conducted using Wilcoxon signed rank-sum test.

We conducted all statistical analyses in the SPSS Version 26.0 (IBM, Chicago, IL) and R software environment (2020).[2] For models implemented in the R software environment, we used the following packages for different modelling frameworks: (1) *nlme* for generalised least squares model (https://cran.r-project.org/web/packages/nlme) and (2) *lme4* for mixed effects logistic regression model (https://cran.r-project.org/web/packages/lme4).

References

1. Pinheiro JC, Bates DM. Mixed-effects models in S and S-PLUS New York. NY: Springer. 2000.

2. Team RC. R Core Team. R: A language and environment for statistical computing. Foundation for Statistical Computing. 2013.