PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Preventive effect of oral magnesium in post-mastectomy pain: protocol for a randomized, double-blind, controlled clinical trial
AUTHORS	MOREL, Véronique; JOLY, Dominique; VILLATTE, Christine; Pereira, Bruno; PICKERING, Gisèle

VERSION 1 – REVIEW

REVIEWER	Ewan McNicol, MS PharmD Clinical Pharmacist, Associate Professor of Anesthesiology, Tufts Medical Center and Tufts University School of Medicine, USA
REVIEW RETURNED	25-Oct-2017

GENERAL COMMENTS	This a generally well-written, clear and concise protocol. The methodology appears sound. The manuscript would benefit from minor editing by a native English speaker. Some terms are confusing or not routinely used in scientific manuscripts. Introduction The references appear to be out of order in places. Page 5, Line 2: “Preclinical and clinical pain studies have reported the controverted curative effect of magnesium on pain with satisfactory15-30 and mitigated results.31-40” Suggest something like “Preclinical and clinical pain studies with magnesium have produce equivocal results with some studies demonstrating analgesic efficacy15-30, while others did not.31-40” Methods and Analysis The term D0-21 is confusing – it suggests a range of days. I would suggest using D-21. I would also suggest not using NPS as an abbreviation for the numeric pain scale, as NPS is normally used as an abbreviation for the Neuropathic Pain Scale. The authors might consider NRS instead (numeric rating scale). Adverse events: Are questions about adverse events in the diary open ended or are specific adverse events listed with yes/no options? Is severity assessed? Will investigators assess causality? Why are adverse events not assessed until two weeks after initiating interventions? Treatment: Please explain why magnesium is started two weeks before surgery. Exclusion criteria: Why are patients receiving quinidine or L-dopa excluded? Blinding: Do the placebo capsules appear identical to the active intervention? If not, what steps will be taken to ensure investigators do not see the different capsules? Primary endpoint: Please clarify exactly what is being measured on the pain scale: pain at that moment; average pain over the last 24

	hours; worst pain over the last 24 hours; etc? Also, please explain why pain at one month was chosen as the primary endpoint. Persistent post-surgical pain is usually defined as pain lasting at least 3 months postoperatively. Statistical analysis Will axillary dissection be incorporated in the multivariate analysis? The investigators mention that a sensitivity analysis will be considered to measure the impact of missing data. How will missing data be handled – BOCF, LOCF, etc? Will an ITT analysis be performed for both efficacy and safety? References References 3-5 do not appear to referred to in the text

REVIEWER	Coquerel A University of Caen - Normandie, Medical pharmacology Dpt, 14032 Caen cedex, France
REVIEW RETURNED	01-Nov-2017

GENERAL COMMENTS	I agree the publication of this paper

REVIEWER	Robert J. McCarthy Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
REVIEW RETURNED	15-Nov-2017

GENERAL COMMENTS	The sample size analysis is incomplete. Estimations of the NPS at M1 is not provided and there is no mention of the statistical method used for the estimation. In the statistical analysis section it appears that the investigators intend to use a Student's t- test of Mann-Whitney U test for the primary analysis, but then mention using analysis of covariance with the baseline average NPS as a covariate. Which is the planned method? This need to be considered in the sample size estimate. In addition the authors frequently suggest in the introduction section that magnesium is being used to prevent pain. A reduction in the NPS (which I assume is at rest, but never stated) of 1 does not suggest prevention of pain. Please reconsider the use of prevent in the introsuction section. How many patients do you expect to have pain at 1 month? This is never stated. I believe the study is substantially underpowered. We have recently completed a study of chronic pain after mastectomy and found mean pain scores of 1 (0 to 3) at 3 months following mastectomy (Pain Practice 2017; PMID:28691269). I do not believe that the sample will be adequate to address the primary outcome.

VERSION 1 – AUTHOR RESPONSE

Reviewer reports:

Reviewer: 1
Reviewer Name: Ewan McNicol, MS PharmD
Institution and Country: Clinical Pharmacist, Associate Professor of Anesthesiology, Tufts Medical Center and Tufts University School of Medicine, USA Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below. This a generally well-written, clear and concise protocol. The methodology appears sound. The manuscript would benefit from minor editing by a native English speaker. Some terms are confusing or not routinely used in scientific manuscripts.

• Introduction
The references appear to be out of order in places.
Page 5, Line 2: “Preclinical and clinical pain studies have reported the controverted curative effect of magnesium on pain with satisfactory15-30 and mitigated results.31-40” Suggest something like “Preclinical and clinical pain studies with magnesium have produce equivocal results with some studies demonstrating analgesic efficacy15-30, while others did not.31-40”

We thank the reviewer for this remark and four references have been reindexed: two have been replaced by studies showing satisfactory results (Bhatia et al., 2004; Tramer et al., 1996) and two, in the reference group showing negative results (Pfaffenrath et al., 1996; Wang et al., 2003).

• Methods and Analysis
The term D0-21 is confusing – it suggests a range of days. I would suggest using D-21.

I would also suggest not using NPS as an abbreviation for the numeric pain scale, as NPS is normally used as an abbreviation for the Neuropathic Pain Scale. The authors might consider NRS instead (numeric rating scale).

Taking account of the reviewer’s comment, these modifications have been done on page 5 and page 13 of the revised manuscript.

Adverse events: Are questions about adverse events in the diary open ended or are specific adverse events listed with yes/no options? Is severity assessed? Will investigators assess causality? Why are adverse events not assessed until two weeks after initiating interventions?

Concerning adverse events, questions are open ended and they are assessed from the beginning of the study (2 weeks before surgery) to the end. Patients will be called once a week to record adverse events starting the first week when the patient starts the treatment until the week before the last visit of the study. Patients may call at any time if an adverse event is observed. Severity and causality will be assessed according to French Pharmacovigilance criteria. Adverse events will be recorded daily in the diary from the day of surgery up to 3 months.

Treatment: Please explain why magnesium is started two weeks before surgery.

This study is a mirror study of a trial using memantine starting two weeks before surgery. This randomized, pilot clinical trial included 40 women undergoing mastectomy in the Oncology Department, University Hospital, Clermont-Ferrand, France showed that patients receiving memantine had at three months a significant difference in post-mastectomy pain intensity, less rescue analgesia and a better emotional state compared to placebo group. An improvement of pain symptoms induced by cancer chemotherapy was also reported (Morel et al., 2016). The aim is to obtain a steady state of systemic blood Magnesium.

Exclusion criteria: Why are patients receiving quinidine or L-dopa excluded?

The magnesium used in this study is “chlorure of magnesium” and with this form of magnesium, quinidine is contra-indicated because this may lead to an increase in the plasma levels of quinidine and the risk of overdose (reduction of renal excretion of quinidine by alkalinization of the urine).

Concerning L-dopa, it may cause biological abnormalities at hepatic or renal level and could bias the interpretation of biological results particularly for the dosage of creatinine that will be performed in this study.

Blinding: Do the placebo capsules appear identical to the active intervention? If not, what steps will be taken to ensure investigators do not see the different capsules?

Capsules and packaging of placebo and magnesium appear identical. Furthermore, in order to maintain blinding, a nurse independent from the protocol gives the treatment to the patient. The person who performs questionnaires and data analyses are different and are not involved in other aspects of the protocol.

Primary endpoint: Please clarify exactly what is being measured on the pain scale: pain at that moment; average pain over the last 24 hours; worst pain over the last 24 hours; etc? Also, please explain why pain at one month was chosen as the primary endpoint. Persistent post-surgical pain is usually defined as pain lasting at least 3 months postoperatively.

The primary endpoint is the average pain intensity (numerical rating scale) evaluated over 5 days before the post-surgery visit at one month in the magnesium and placebo groups. This precision has been added on page 7 line 22 of the revised manuscript.
Furthermore, the aim of this study is to evaluate if an oral administration of magnesium before mastectomy could induce a decrease of post-operative pain; the primary endpoint was at one month post-surgery in order to evaluate pain continuum and have data in case patients could not tolerate Mg for longer. Persistent post-operative pain will be also evaluated at 3 months post-mastectomy.

• Statistical analysis
Will axillary dissection be incorporated in the multivariate analysis?

We thank the reviewer for the relevant comment. So, axillary dissection has been added as covariate in the multivariate analysis (page 12, line 13 of the revised manuscript).

The investigators mention that a sensitivity analysis will be considered to measure the impact of missing data. How will missing data be handled – BOCF, LOCF, etc?

We thank the reviewer for the helpful comment. We completely agree that it is necessary to give more information about method used to take into account missing data even if the approach will depend of the nature of missing data (missing or not at random). According to reviewer's comment, we have completed this part of Statistical Section: "A sensitivity analysis will be performed to measure the impact of missing data and to assess the problem caused by missing longitudinal data at M3. The nature of missing data will be studied (missing at random or not). According to this, the most appropriate approach to the imputation of missing data will be proposed: multiple imputation, maximum bias (last observation carried forward vs baseline observation carried forward) or estimation proposed by Verbeke and Molenberghs for repeated data.52" (page 12 of the revised manuscript).

Will an ITT analysis be performed for both efficacy and safety?

We propose to consider ITT analysis only for efficacy.

• References
References 3-5 do not appear to referred to in the text

We thank the reviewer for this remark and modified accordingly on page 4 of the revised manuscript.

Reviewer: 2
Reviewer Name: Coquerel A
Institution and Country: University of Caen - Normandie, Medical pharmacology Dpt, 14032 Caen cedex, France Please state any competing interests or state ‘None declared’: none declared

Please leave your comments for the authors below I agree the publication of this paper

Reviewer: 3
Reviewer Name: Robert J. McCarthy
Institution and Country: Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA
Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below. The sample size analysis is incomplete. Estimations of the NPS at M1 is not provided and there is no mention of the statistical method used for the estimation. In the statistical analysis section it appears that the investigators intend to use a Student's t- test of Mann-Whitney U test for the primary analysis, but then mention using analysis of covariance with the baseline average NPS as a covariate. Which is the planned method? This need to be considered in the sample size estimate.

We thank the reviewer for the comment which give us the opportunity to improve the quality of presentation concerning sample size estimation and primary statistical analysis.

As discussed by Vickers and Altman, the use of ANCOVA depends on the intensity of correlation between baseline and follow up scores: “If the treatment is effective the statistical significance of the treatment effect by the two methods will depend on the correlation between baseline and follow up scores. If the correlation is low using the change score will add variation and the follow up score is more likely to show a significant result. Conversely, if the correlation is high using only the follow up score will lose information and the change score is more likely to be significant. It is incorrect, however, to choose whichever analysis gives a more significant finding.” Without information concerning correlation between baseline and follow up scores, it is proposed to perform the analysis of the primary objective using Student t-test or Mann-Whitney test. If appropriate (high correlation between baseline and follow up scores), the analysis of covariance with the baseline average NPS as a covariate will be proposed as multivariable analysis, as described by Vickers and Altman: “Lastly, analysis of covariance is a type of multiple regression and can be seen as a special type of adjusted analysis. The analysis can thus be expanded to include additional prognostic variables (not necessarily continuous), such as age and diagnostic group.”

According to these arguments (notably due to lack of information concerning the correlation between baseline and follow up scores), sample size estimation has been performed using Stata software (version 13, StataCorp, College Station, US) with command sampsi based on usual sample size estimation (Machin D, Campbell MJ, Tan SB, Tan SH (2009) Sample size tables for clinical studies. 3rd ed. Chichester: Wiley-Blackwell).
with:
– Type I error α at 5% (two-sided): Z1-α=1.96
– Type II error β at 10%: Z1-β=1.28
– Δ, difference on the knowledge score between groups, at least 1 points
– σ, standard-deviation of knowledge score, equals 1.5

According to helpful reviewer’s comment, we have modified Statistical Section: (page 11-12 of the revised manuscript)

Sample size calculation

Sample size estimation has been performed using Stata software (version 13, StataCorp, College Station, US) with command sampsi based on usual sample size estimation (Machin D, Campbell MJ, Tan SB, Tan SH (2009) Sample size tables for clinical studies. 3rd ed. Chichester: Wiley-Blackwell). Considering the literature, the prevalence of Post-mastectomy Pain is 20% (Smith et al., 1999; Stevens et al., 1995). However, these data may vary depending on demographic, psychological and medical/surgical factors (Schreiber et al., 2014) and will be taken into consideration in this study. We assume it will be 20% as well. The number of subjects required is 100 patients with breast cancer undergoing total mastectomy (50 in each group). The minimum δ difference in numerical pain scale between magnesium and placebo groups at M1 is estimated at 1.0 and σ standard deviation at 1.5 with $\alpha = 0.05$ two-sided type I error and $\beta = 0.10$.

Statistical analysis

Statistical analyses will be performed with Stata software (version 13; StataCorp, College Station, US). Concerning the primary objective, comparison between the randomized groups will be performed using the Student test or the Mann and Whitney test (if the conditions for validity of the Student test are not respected, normality verified by Shapiro-Wilk and homoscedasticity by Fisher-Snedecor test). If a high correlation between baseline and follow up scores is highlighted, an analysis of covariance with the baseline average NPS as a covariate will be proposed as multivariable analysis, as proposed by Vickers and Altman (Vickers and Altman, 2001). This analysis can thus be expanded to include additional prognostic variables. The confounding factors likely to influence the primary endpoint (para-vertebral block, breast reconstruction with latissimus dorsi muscle flap, axillary dissection) will be taking into account in multivariate regression analysis. Concerning anesthesia, it is generally standardized and the authorized treatment will be noted. There will be a systematic adjustment for the main analysis. The analysis of repeated data (at the inclusion, M1 and M3) will be carried out by mixed models which allow to consider, on the one hand, time, group and their interaction time x group as fixed effects and on the other hand, the within and between subject variability. A sensitivity analysis will be considered to measure the impact of missing data and to assess the problem caused by missing longitudinal data at M3. A sensitivity analysis will be performed to measure the impact of missing data and to assess the problem caused by missing longitudinal data at M3. The nature of missing data will be studied (missing at random or not). According to this, the most appropriate approach to the imputation of missing data will be proposed: multiple imputation, maximum bias (last observation carried forward vs baseline observation carried forward) or estimation proposed by Verbeke and Molenberghs for repeated data.52”

In addition the authors frequently suggest in the introduction section that magnesium is being used to prevent pain. A reduction in the NPS (which I assume is at rest, but never stated) of 1 does not suggest prevention of pain. Please reconsider the use of prevent in the introduction section.

Taking account the reviewer’s comment, the use of “prevent” has been reconsidered and some sentences have been modified in the introduction section on page 5, lines 3-9 of the revised manuscript.

How many patients do you expect to have pain at 1 month? This is never stated.

If we based on considering the literature, the prevalence of Post-mastectomy Pain is 20% (Smith et al., 1999; Stevens et al., 1995). However, these data may vary depending on demographic, psychological and medical/surgical factors (Schreiber et al., 2014) and will be taken into consideration in this study. This element has been added on page 11, lines 21-23.

Post-operative sensations reported by patients can be transient or long-lasting and can include pain, phantom sensations and sensory loss. Data of the literature reported that an optimal management of acute postoperative pain may also reduce the development of chronic postsurgical pain (Macrae, 2001). Up to 65% of patients undergoing breast cancer surgery develop CPSP, a complaint that is associated with reduced quality of life and can last for several years (Kehlet et al., 2006; Hayes et al.,

2002). Furthermore, our previous study underlines the importance of a preventive management in order to limit the development of persistent post-operative pain (Morel et al., 2016).

I believe the study is substantially underpowered. We have recently completed a study of chronic pain after mastectomy and found mean pain scores of 1 (0 to 3) at 3 months following mastectomy (Pain Practice 2017; PMID:28691269). I do not believe that the sample will be adequate to address the primary outcome.

We are grateful to give us information about your experience. However, we can argue several reasons to be confident in this study:
1) The statistical power equals 90%, greater than 80%. As discussed above, without information concerning correlation between baseline and follow up scores, it is proposed to perform the analysis of the primary objective using Student t-test or Mann-Whitney test. If appropriate (high correlation between baseline and follow up scores), the analysis of covariance with the baseline average NRS as a covariate will be proposed as multivariable analysis. As discussed by Vickers and Altman, “an advantage of analysis of covariance is that it generally has greater statistical power to detect a treatment effect than the other methods. For example, a trial with a correlation between baseline and follow up scores of 0.6 that required 85 patients for analysis of follow up scores, would require 68 for a change score analysis but only 54 for analysis of covariance.”
2) Finally, we have read with a great interest the reviewer’s article suggestion (The Effect of Intraoperative Systemic Lidocaine on Postoperative Persistent Pain Using Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials Criteria Assessment Following Breast Cancer Surgery: A Randomized, Double-Blind, Placebo-Controlled Trial. Pain Pract. 2017 Jul 10). The study was a randomized, double-blinded, placebo-controlled, clinical trial. Patients were evaluated at 3 and 6 months for the presence of chronic persistent postsurgical pain. Perioperative infusion of lidocaine has been reported to decrease the incidence of postsurgical pain at 3 and 6 months following mastectomy using dichotomous (yes/no) scoring. This study has been planned using a binary primary outcome whereas our study is based on pain intensity evaluated by NRS, ranged from 0 no pain to 10 maximal tolerable pain. With the same number of patients (one hundred forty-eight) those proposed in this study, a minimal difference of 0.8 points between groups should be highlighted (for a statistical power at 90%); such difference does not seem sufficiently relevant.

VERSION 2 – REVIEW

REVIEWER	Robert J. McCarthy Northwestern University, Chicago, Illinois
REVIEW RETURNED	15-Dec-2017

GENERAL COMMENTS	The title states that the intervention is targeted at mastectomy-induced neuropathic pain but the primary outcome is the NRS pain score. A difference in 1 in the NRS pain score may have nothing to do with the incidence

REVIEWER	Ewan McNicol, MS PharmD Clinical Pharmacist, Associate Professor of Anesthesiology, Tufts Medical Center and Tufts University School of, Medicine, USA
REVIEW RETURNED	02-Jan-2018

GENERAL COMMENTS	I still think the manuscript would benefit from minor editing by a native English speaker, but perhaps this could be performed by the editorial team? Other than that, the authors have adequately addressed all of my concerns. Thank you.

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer reports:
• Reviewer: 3
Reviewer Name: Robert J. McCarthy
Institution and Country: Northwestern University, Chicago, Illinois Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below: The title states that the intervention is targeted at mastectomy-induced neuropathic pain but the primary outcome is the NRS pain score.

Taking account of the comments of the Editor and reviewer, the title has been modified: "Preventive effect of oral magnesium in post-mastectomy pain: protocol for a randomized, double-blind, controlled clinical trial".

A difference in 1 in the NRS pain score may have nothing to do with the incidence.

We thank the reviewer for this remark and "incidence" has been modified in our previous response to reviewers by the term "occurrence":

"I believe the study is substantially underpowered. We have recently completed a study of chronic pain after mastectomy and found mean pain scores of 1 (0 to 3) at 3 months following mastectomy (Pain Practice 2017; PMID:28691269). I do not believe that the sample will be adequate to address the primary outcome".

We are grateful to give us information about your experience. However, we can argue several reasons to be confident in this study:
1) The statistical power equals 90%, greater than 80%. As discussed above, without information concerning correlation between baseline and follow up scores, it is proposed to perform the analysis of the primary objective using Student t-test or Mann-Whitney test. If appropriate (high correlation between baseline and follow up scores), the analysis of covariance with the baseline average NRS as a covariate will be proposed as multivariable analysis. As discussed by Vickers and Altman, "an advantage of analysis of covariance is that it generally has greater statistical power to detect a treatment effect than the other methods. For example, a trial with a correlation between baseline and follow up scores of 0.6 that required 85 patients for analysis of follow up scores, would require 68 for a change score analysis but only 54 for analysis of covariance."
2) Finally, we have read with a great interest the reviewer's article suggestion (The Effect of Intraoperative Systemic Lidocaine on Postoperative Persistent Pain Using Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials Criteria Assessment Following Breast Cancer Surgery: A Randomized, Double-Blind, Placebo-Controlled Trial. Pain Pract. 2017 Jul 10). The study was a randomized, double-blinded, placebo-controlled, clinical trial. Patients were evaluated at 3 and 6 months for the presence of chronic persistent postsurgical pain. Perioperative infusion of lidocaine has been reported to decrease the occurrence of postsurgical pain at 3 and 6 months following mastectomy using dichotomous (yes/no) scoring. This study has been planned using a binary primary outcome whereas our study is based on pain intensity evaluated by NRS, ranged from 0 no pain to 10 maximal tolerable pain. With the same number of patients (one hundred forty-eight) those proposed in this study, a minimal difference of 0.8 points between groups should be highlighted (for a statistical

power at 90%); such difference does not seem sufficiently relevant.

• Reviewer: 1
Reviewer Name: Ewan McNicol, MS PharmD
Institution and Country: Clinical Pharmacist, Associate Professor of Anesthesiology, Tufts Medical Center and Tufts University School of Medicine, USA Please state any competing interests or state 'None declared': None Declared

Please leave your comments for the authors below I still think the manuscript would benefit from minor editing by a native English speaker, but perhaps this could be performed by the editorial team? Other than that, the authors have adequately addressed all of my concerns. Thank you.