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Complete List of Authors:	MOREL, Véronique; Centre Hospitalier Universitaire de Clermont-Ferrand, CPC/CIC Inserm 1405, Bât 3C JOLY, Dominique; Centre Jean Perrin VILLATTE, Christine; Centre Jean Perrin Pereira, Bruno; University Hospital CHU Clermont-Ferrand, PICKERING, Gisèle; Centre Hospitalier Universitaire de Clermont-Ferrand, CPC/CIC Inserm 1405, Bât 3C
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Preventive effect of oral magnesium in mastectomy-induced neuropathic pain: a randomized, double-blind, controlled clinical trial

Short running title

Magnesium for neuropathic pain prevention

Trial acronym: MagNet

Véronique Morel[c], Dominique Joly[d], Christine Villatte[d], Bruno Pereira[e], Gisèle Pickering[a,b,c],

[a] Université Clermont Auvergne, Laboratoire de Pharmacologie, Facultés de Médecine/Pharmacie, F-63001 Clermont-Ferrand, France.

[b] Inserm, U1107 Neuro-Dol, Pharmacologie Fondamentale et Clinique de la Douleur, F-63001 Clermont-Ferrand, France.

[c] CHU Clermont-Ferrand, Inserm CIC Inserm 1405, Centre de Pharmacologie Clinique, F-63003 Clermont-Ferrand, France

[d] CHU Clermont-Ferrand, Centre Jean Perrin, Centre de Lutte contre le Cancer, 58 rue Montalembert, F-63000 Clermont-Ferrand, France.

[e] CHU de Clermont-Ferrand, Délégation Recherche Clinique & Innovation - Villa annexe IFSI, 58 Rue Montalembert, F-63003 Clermont-Ferrand cedex, France.

Correspondence

Véronique MOREL, Centre de Pharmacologie Clinique, Bâtiment 3C, CIC Inserm 1405, CHU Clermont-Ferrand, BP 69, F-63003 Clermont-Ferrand Cedex 1, France

Tel: (+33) 4 73 17 84 11 / Fax: (+33) 4 73 17 84 12

E-mail address: v_morel@chu-clermontferrand.fr

ABSTRACT

Introduction: Breast cancer affects one in ten women worldwide and mastectomy is a cause of chronic pain with neuropathic characteristics. *N*-methyl-D-aspartate receptor (NMDAR) antagonists such as ketamine, memantine, dextromethorphan or magnesium, by blocking NMDAR are used for refractory pain. Oral memantine has been shown to prevent post-mastectomy pain, cognitive impact and maintain quality of life. In a similar fashion, this present study will evaluate the preventive effect of oral magnesium, given upstream of the mastectomy, on neuropathic pain development. As a physiological blocker of NMDAR, magnesium could be an interesting candidate to prevent post-operative pain and associated comorbidities, including cognitivo-emotional disorders, multiple analgesics consumption and impaired quality of life.

Methods and analysis: A randomized, double-blind, controlled clinical trial (NCT 03063931) includes 100 women with breast cancer undergoing mastectomy at the Oncology Hospital, Clermont-Ferrand, France. Magnesium (100 mg/day; n=50) or placebo (n=50) is administered for six weeks starting two weeks before surgery. Intensity of pain, cognitivo-emotional function and quality of life are evaluated with questionnaires. The primary endpoint is pain intensity on a (0-10) numerical rating scale at 1 month post-mastectomy. Data analysis is performed using mixed models and the tests are two-sided, with a type I error set at α=0.05.

Ethics and dissemination: The study protocol and the informed consent have been approved in December 2016 by the French Research Ethics Committee (South East VI Committee). Results will be communicated in different congress and published in international review.

Trial registration number: NCT03063931

Keywords

Magnesium, NMDA receptor, breast cancer, mastectomy, neuropathic Pain

Abbreviations

NP, Neuropathic Pain; NMDAR, *N*-Methyl-D-Aspartate receptor

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Authors' contributions

GP is the overall study principal investigator; she participated in the conception and study design and contributed to the writing of the study protocol and the drafting and editing of this manuscript. DJ, CV and BP all participated in the study design. BP contributed to the writing of the study protocol and carried out all statistical calculations and wrote the statistical paragraph in the study protocol. He contributed with GP and VM to the drafting and editing of this manuscript. All authors read and approved the final manuscript.

INTRODUCTION

Breast cancer is the most common cancer in women worldwide and the lifetime probability of developing breast cancer is 12.3%, approximately 1 in 8.[1] Mastectomy, chemotherapy and radiotherapy play an important role in the development of neuropathic pain. Neuropathic pain is defined as pain related to a lesion or disease affecting the somatosensory system. The mechanisms responsible for spinal hyperexcitability include the activation of central and peripheral *N*-methyl-D-aspartate receptor (NMDAR). These play an ubiquitous role in pain central sensitization and in many other functions like memory and learning.[6 7] Neuropathic pain is associated with the development of a number of comorbidities including cognitive-emotional and sleep disorders.[8] Post-mastectomy pain may be reported in the anterior thorax, armpit, upper arm, and edema, sensory dysfunction, neuroma emergence, numbness in the arm contribute to the pain syndrome that affects 20 to 68% of the patients.[2] NMDAR antagonists such as ketamine, memantine, dextromethorphan or magnesium by blocking NMDAR may limit or even reverse the painful phenomena and are possible drugs for pain refractory to recommended treatments. [9-12]

With a translational approach, the prophylactic effect of memantine in neuropathic pain was recently demonstrated in animals and in humans.[12 13] In a preclinical pain model (Spinal Nerve Ligation) memantine has been shown to prevent neuropathic pain development when administered a few days before surgery. Molecular biology tests showed a decrease of pTyr^{1472}NR2B at spinal and supraspinal level (insula and hippocampus).[13] The translational clinical study confirmed the beneficial effect of memantine to prevent post-mastectomy pain development, diminish chemotherapy-induced pain symptoms and analgesic consumption, with a better quality of life for at least 6 months after surgery.[12]

Magnesium is a physiological NMDAR antagonist and blocks calcium and potassium channels of the receptor, modulating NMDAR activation with very few side-effects.[14]

Preclinical and clinical pain studies have reported the controverted curative effect of magnesium on pain with satisfactory[15-30] and mitigated results.[31-40] No study has so far focused on the preventive effect of several weeks oral administration of magnesium on post-operative pain and more specifically in post-operative pain related to breast cancer surgery.

In the present study, magnesium will be administered before surgery in order to evaluate its preventive properties on pain development, cognition, emotion and quality of life during three months after mastectomy. The primary objective is to evaluate if magnesium administered two weeks before and 4 weeks after mastectomy could prevent pain development at one month post-mastectomy compared to placebo. The secondary objectives are the evaluation of pain intensity, analgesic concomitant medications, cognitive-emotional function, quality of life and sleep one and three months after mastectomy

METHODS AND ANALYSIS

Study setting

A randomized, placebo-controlled, double-blind clinical trial will be conducted in the Oncology Hospital, Clermont-Ferrand, France, in 100 women undergoing total mastectomy for breast cancer. The study has been approved in December 2016 by the regional Ethics Committee and registered on February 24 2017 at "http://www.clinicaltrials.gov" (NCT03063931). Three weeks before surgery ($D_{0\text{-}21}$), patients will meet the medical team and the physician will explain to the patient the protocol, the objectives of the study and the different questionnaires and tests that will be carried out in order for the patient to give her written informed consent. If necessary, a sufficient time for reflection will be granted. After having given informed consent, women will rate their pain on the numerical pain scale (NPS) and complete the cognition, emotion, quality of life and sleep questionnaires. A blood test will be performed in order to dose the level of magnesium and creatinine, and participants will be

randomized in two parallel groups: magnesium (n=50) or placebo (n=50). Patients will be contacted fifteen days before surgery in order to be reminded them to start their treatment. Magnesium or placebo (lactose) will be given orally for six weeks starting two weeks before surgery. Magnesium will be given at the dose of 100 mg/day (2 tablets of 50 mg/day once a day). Endpoints will be reassessed at one (M1) and three months (M3) post-mastectomy. Patients will be called once a week by phone in order to maintain a good compliance and to verify they do not develop adverse events. Finally, a booklet to monitor drug intake and adverse events will be completed daily by the patient for 3 months from the day of surgery (D_0). Detailed information on the present study is summarized in Figure 1.

Inclusion criteria

1. Patient is at least 18 years old.
2. Patient with a diagnosis of breast cancer and with planned total mastectomy with or without axillary dissection.
3. Patient with no change of treatment and diet.
4. Patient able to understand and agreeing to follow the study protocol.

Exclusion criteria

1. Patient with any magnesium contraindication: hypersensitivity to magnesium chloride or to any of the excipients.
2. Patient with a magnesemia of more than 1.05 mmol/L.
3. Patient with severe renal insufficiency and with a renal clearance of less than 30 mL/min.
4. Patient with an addiction to alcohol as diagnosed by the investigator.
5. Patient with diabetes (Type I and II).
6. Patient receiving treatment with quinidine or L-Dopa.

7. Patient in childbearing age, with no effective contraceptive method, pregnancy or lactation,
8. Patient enrolled in another clinical trial.
9. Patient with an inability to comply with the requirements of the protocol.

Intervention

Treatment group

The treatment group will receive magnesium during six weeks starting two weeks before surgery. Patients in the magnesium group should take once a day 100 mg/day ((2 x 50 mg) of low dose continuous release magnesium stored in opaque white bottles in order to maintain double blinding (CHRONOMAG®, FJ Life Sciences).

Control group

Patients will receive once a day two tablets of placebo (lactose) during six weeks starting two weeks before mastectomy.

Magnesium and packaging will be provided by FJ Life Sciences Society. Placebo will be prepared, conditioned and released in the Hospital Central pharmacy by one qualified person according to good manufacturing principles. The number of tablets in each dispensed container will be verified and recounted at the end of the treatment by two persons totally independent of the protocol.

Outcome evaluation

The primary endpoint will be the pain intensity evaluation by NPS in magnesium and placebo groups at M1. The scale ranges from 0 no pain to 10 maximal tolerable pain.

The following secondary endpoints will be evaluated at the screening visit, M1 and M3: 1) pain evaluation with NPS and the McGill pain questionnaire;[41] 2) neuropathic pain questionnaire with the neuropathic pain in four questions (DN4) and the Neuropathic Pain

Symptom Inventory questionnaire (NPSI);[42 43] 3) cognition with the Trail Making Test (TMT) and the Functional Assessment of Cancer Therapy-Cognitive Function (FACT-COG);[44 45] 4) anxiety and depression with the Depression Anxiety Stress Scale (DASS);[46] 5) quality of life with European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 items (EORTC QLQ-C30) and the Patient Global Impression of Change (PGIC) and 6) sleep with the Pittsburgh Sleep Quality Index (PSQI).[47-49] At the inclusion visit, M1 and M3, blood and urinary concentrations of magnesium will be measured. A summary of the evaluations for one patient is reported in table 1.

- *McGill pain questionnaire*[41]

This questionnaire allows to qualify pain experience during the last 48 hours. It has fifty eight qualifiers divided into sixteen items (A to P). Each qualifier is rated from 0 to 4, where 0 = absent, 1 = low, 2 = moderate, 3 = strong, 4 = very strong. The score is divided between two subclasses, sensory subclass (items A to I) and emotional subclass (items J to P).

- *Neuropathic Pain in 4 questions questionnaire (DN4)*[42]

DN4 is a clinical tool for the diagnosis of neuropathic pain. This questionnaire has four questions divided into 10 items related to the interview (ie, symptoms) and to the sensory examination (ie, signs). The investigator asks and examines the patient and notes a response "no" or "yes" for each item: "yes" is scored as "1" and "no" is scored as "0". The sum of scores gives the total score of the patient (/10). DN4 is considered as positive if the patient obtains a score ≥ 4/10.

- *Neuropathic Pain Symptom Inventory (NPSI)*[43]

NPSI is a self-questionnaire and includes 10 pain descriptors. Intensity is rated on 0-10 numerical scales and two temporal items are designed to assess spontaneous ongoing pain duration and the number of pain paroxysms over 24h. This questionnaire discriminates 5

distinct clinically relevant dimensions: spontaneous burning pain, spontaneous deep pain, paroxysmal pain, evoked pain, and paresthesia/dysesthesia.

- *Trail Making Test (TMT)*[44]

This non-verbal cognitive test assesses the ability of speed, executive function, attention, concentration, visual perceptual speed. In Part A, circles are numbered from 1 to 25 and the patient must connect with lines the numbers in ascending order (1-2-3-4, etc.). In Part B, the circles contain numbers from 1 to 13 and letters from A to L, the patient must connect the circles with lines but alternating numbers and letters (1A-2B -3C, etc.). The patient must connect the circles as quickly as possible for both parts of the test, without lifting the pen from the paper. The TMT B additionally provides an estimate of mental flexibility.

- *Functional Assessment of Cancer Therapy-Cognitive Function (FACT-COG)*[45]

This self-report questionnaire has been validated with cancer patients and assesses the impairment of cognitive ability and its impact on the patient's quality of life. It consists in 37 items assessing memory, attention, concentration, language and thinking abilities. The items are rated using a 5-point Likert scale. The FACT-COG takes into consideration the functional implication of cognitive impairment, the deficits observed by other people, the changes in cognitive function over time, and their impact on the patient's quality of life.

- *Depression Anxiety Stress Scale*[46]

The DASS is a 42-item self-report instrument designed to measure the three related negative emotional states of depression, anxiety and tension/stress. The DASS Depression focuses on reports of low mood, motivation, and self-esteem, DASS-anxiety on physiological arousal, perceived panic, and fear, and DASS-stress on tension and irritability. A respondent indicates on a 4-point scale the extent to which each of 42 statements applied over the past week. A printed overlay is used to obtain total scores for each subscale. Higher scores on each subscale indicate increasing severity of depression, anxiety or stress.

- *European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 items (EORTC QLQ-C30)*[47]

This questionnaire assesses the quality of life of cancer patients. It is divided in 9 subscales consisting of several items: 5 subscales measuring functional status (physical, role, social, emotional, cognitive), three subscales measuring symptoms (fatigue, pain, nausea and vomiting) and a global subscale of quality of life and health. Finally, six items/isolated symptoms, covering cancer symptoms and frequent side effects of cancer therapies are also included in the EORTC QLQ-C30.

- *Patient Global Impression of Change*[48]

This is a 7-point self-reported numerical scale used to assess what the change in their condition following treatment meant to the patient.

- *Pittsburgh Sleep Quality Index*[49]

This questionnaire consists of 19 items and is used to measure sleep quality. It consists of 7 domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication and daytime dysfunction.

Recruitment and Randomization

Three weeks before mastectomy, when the informed consent is signed, blood samples (magnesium and creatinine concentrations) and questionnaires will be performed. Then, patients will be randomized in the magnesium (n=50) or placebo group (n=50). Treatment allocation will follow a predetermined randomization list and will be carried out by a person totally independent from the protocol. The randomization sequence will be generated using random blocks. Treatments will be packed in a similar opaque bottle covered with an identical label indicating batch number, expiry date and sponsor code with no indication of the name of the drug. In order to maintain blinding, the physician who evaluated pain could not guess allocation at any time and would not meet the patient again in the course of the trial.

Sample size calculation

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 situation 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). The recommendations proposed by Vickers and Altman (Vickers and Altman, 2001) will be implemented.[50] Thus, a covariance analysis considering the measure of average pain at inclusion as a covariate will be proposed. The confounding factors likely to influence the primary endpoint (para-vertebral block, breast reconstruction with latissimus dorsi muscle flap) 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. Estimation methods developed by Verbeke and Molenberghs will be proposed.[51]

Data handling and record keeping

All original records such as consent forms, Case Report Forms, questionnaires and pain diary will be archived at trial sites for 15 years. The database file will be anonymized and maintained also for 15 years. The monitoring will be performed by a clinical research associated independent from the protocol. Then, the monitored case report forms will be transferred to the Data Management Center (CIC-Inserm 1405, Clermont-Ferrand).

Duration of the study

The duration of treatment will be 42 days. The total duration of participation per patient will be of 14 weeks. The protocol will include 4 visits ($D_{0\text{-}21}$/D_0/M1/M3) including a period of hospitalization per patient. Treatment will be given daily starting two weeks before surgery and maintained four weeks after. The recruitment will start in June 2017. The total duration of the study is estimated at two years.

ETHICS AND DISSEMINATION

The study received approval by the French Research Ethics Committee on December 2016 (ID-RCB n° 2016-A01749-42). The trial is registered in ClinicalTrials.gov (trial n° NCT03063931). Each patient meeting the inclusion criteria will sign a Consent Form after receiving oral and written information. After agreement between all investigators, data will be disclosed and results will be communicated in different congress and published in international review.

DISCUSSION

Following successful results obtained with prophylactic memantine in neuropathic pain development,[12 13] this study aims at assessing magnesium treatment in a similar protocol to prevent neuropathic pain induced by mastectomy. In breast cancer surgery, clinical studies using magnesium have focused so far on the qualitative and emotional aspects of pain rather

than on the intensity of pain itself.[52] Magnesium in neuropathic pain alleviation has shown controverted results.[19-36]Magnesium has been shown to modulate the limbic system *via* NMDAR and these brain areas are known to be involved in emotion and pain.[53] It is therefore essential to evaluate concomitantly magnesium effect on pain and also on cognitive-emotional and sleep aspects. Magnesium deprivation may affect cognition and sleep quality. Preclinical findings showed that an increase in brain magnesium enhances both short-term synaptic facilitation and long-term potentiation and improves learning and memory functions.[54] In human, a study showed that preeclamptics patient receiving magnesium had better attention and working memory performance both before and after delivery compared to controls.[55] Furthermore, a review reported the relationship between low level of magnesium, stress and cognitive difficulties such as lack of concentration and difficulties in learning.[56] Concerning the impact of magnesium on sleep, a placebo-controlled, randomized cross-over study performed in 12 older participants showed that magnesium supplementation significantly reversed electroencephalogram changes, including decreased slow wave sleep, that may occur during aging.[57] Furthermore, a double blind trial reported that intraoperative infusion of magnesium led to a significantly better quality of sleep during the post-operative period without any side-effects.[58]

Magnesium is an abundant mineral, naturally present in food and is available as a dietary supplement that is appreciated by patients.[59] It is obtained without prescription and has a favourable risk-benefit balance with few side effects.[59] This molecule is also known to regulate diverse biochemical reactions in the body and is required for energy production, oxidative phosphorylation, and glycolysis. It also plays a role in the active transport of calcium and potassium ions across cell membranes, a process that is important to nerve impulse conduction, muscle contraction, and normal heart rhythm.[60 61] Low blood levels of magnesium have been associated with a number of pathologies including type-2 diabetes, or

cardio-vascular disease.[62] Oral magnesium supplementation is usually well tolerated and gastrointestinal side effects including nausea, vomiting, and diarrhea are usually minor.[62 63]

The pharmaceutical form in this trial provides magnesium chloride, a circulating form of magnesium with a gradual and constant release of low doses of magnesium along the gastro intestinal tract. A recent clinical study (NCT01935570) showed that the dose of 100 mg daily guarantees an optimal absorption of magnesium by the body over a 24-hour period. Furthermore, this form of magnesium does not induce intestinal side effects and is easy to use with a once a day intake.

In conclusion, if magnesium given before and after mastectomy proves its efficacity in neuropathic pain prevention, it could be an excellent prophylactic strategy to prevent post-mastectomy pain symptoms, maintain quality of life and cognitive function and limit comorbidities that accompany breast cancer pathology.

Competing interests

The authors declare that there are no financial or non-financial competing interests neither within the conception nor conduction of the trial.

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Figure 1: Study design

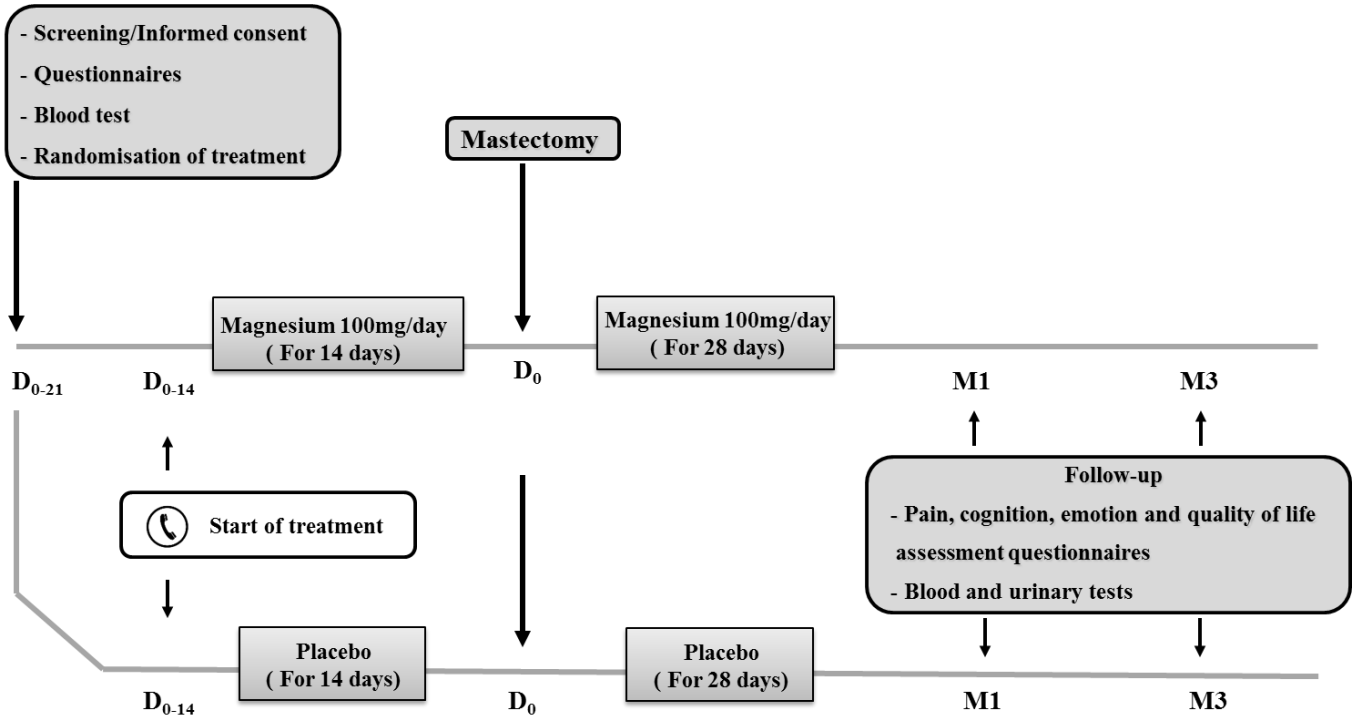

Table 1: Summary of assessments

	STUDY PERIOD				
	Enrolment	Allocation	Post-allocation		Close-out
TIMEPOINT	**D0-21**	**D0-14**	**D0**	**M1**	**M3**
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Allocation		X			
INTERVENTIONS:					
Start of treatment		X			
End of treatment				X	
Surgery			X		
Blood test	X			X	X
Urinary test				X	X
Delivrey pain diary			X		
ASSESSMENTS:					
Numeric Pain Scale (NPS)	X			X	X
Neuropathic Pain four questions (DN4)	X			X	X
Neuropathic Pain Symptom Inventory (NPSI)	X			X	X
McGill Pain questionnaire	X			X	X
Trail Making Test (TMT)	X			X	X
Functional Assessment of Cancer Therapy-Cognitive Function (F	X			X	X
EORTC QLQ-C30	X			X	X
Depression Anxiety Stress Scale (DASS)	X			X	X
Pittsburgh Sleep Quality Index (PSQI)	X			X	X
Patient Global Impression of Change (PGIC)	X			X	X
Concomitant analgesic treatments	X	X	X	X	X
Adverse events			X	X	X

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____2_____
	2b	All items from the World Health Organization Trial Registration Data Set	_____NA____
Protocol version	3	Date and version identifier	_____3_____
Funding	4	Sources and types of financial, material, and other support	_____3_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	__1 and 3_____
	5b	Name and contact information for the trial sponsor	_____3_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____/_____
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____3_____

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	/
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5-6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	/
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	____11_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____11_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	__10_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	__10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	__10-11_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	__10-11_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____/_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	___5-6_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___5-6_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_10-11_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_11_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_11_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_11_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____12_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____/_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____/_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____/_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____12_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____/_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	/
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	/
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12
	31b	Authorship eligibility guidelines and any intended use of professional writers	/
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	/
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	/
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	/

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

BMJ Open

Preventive effect of oral magnesium in mastectomy-induced neuropathic pain: a randomized, double-blind, controlled clinical trial

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Magnesium, NMDA receptor, breast cancer, mastectomy, neuropathic Pain

Revised manuscript

Preventive effect of oral magnesium in mastectomy-induced neuropathic pain: a randomized, double-blind, controlled clinical trial

Short running title

Magnesium for neuropathic pain prevention

Trial acronym: MagNet

Véronique Morel[c], Dominique Joly[d], Christine Villatte[d], Bruno Pereira[e], Gisèle Pickering[a,b,c],

[a] Université Clermont Auvergne, Laboratoire de Pharmacologie, Facultés de Médecine/Pharmacie, F-63001 Clermont-Ferrand, France.

[b] Inserm, U1107 Neuro-Dol, Pharmacologie Fondamentale et Clinique de la Douleur, F-63001 Clermont-Ferrand, France.

[c] CHU Clermont-Ferrand, Inserm CIC Inserm 1405, Centre de Pharmacologie Clinique, F-63003 Clermont-Ferrand, France

[d] CHU Clermont-Ferrand, Centre Jean Perrin, Centre de Lutte contre le Cancer, 58 rue Montalembert, F-63000 Clermont-Ferrand, France.

[e] CHU de Clermont-Ferrand, Délégation Recherche Clinique & Innovation - Villa annexe IFSI, 58 Rue Montalembert, F-63003 Clermont-Ferrand cedex, France.

Correspondence

Véronique MOREL, Centre de Pharmacologie Clinique, Bâtiment 3C, CIC Inserm 1405, CHU Clermont-Ferrand, BP 69, F-63003 Clermont-Ferrand Cedex 1, France

Tel: (+33) 4 73 17 84 11 / Fax: (+33) 4 73 17 84 12

E-mail address: v_morel@chu-clermontferrand.fr

ABSTRACT

Introduction: Breast cancer affects one in ten women worldwide and mastectomy is a cause of chronic pain with neuropathic characteristics. *N*-methyl-D-aspartate receptor (NMDAR) antagonists such as ketamine, memantine, dextromethorphan or magnesium, by blocking NMDAR are used for refractory pain. Oral memantine has been shown to prevent post-mastectomy pain, cognitive impact and maintain quality of life. In a similar fashion, this present study will evaluate the preventive effect of oral magnesium, given upstream of the mastectomy, on neuropathic pain development. As a physiological blocker of NMDAR, magnesium could be an interesting candidate to prevent post-operative pain and associated comorbidities, including cognitivo-emotional disorders, multiple analgesics consumption and impaired quality of life.

Methods and analysis: A randomized, double-blind, controlled clinical trial (NCT 03063931) includes 100 women with breast cancer undergoing mastectomy at the Oncology Hospital, Clermont-Ferrand, France. Magnesium (100 mg/day; n=50) or placebo (n=50) is administered for six weeks starting two weeks before surgery. Intensity of pain, cognitivo-emotional function and quality of life are evaluated with questionnaires. The primary endpoint is pain intensity on a (0-10) numerical rating scale at 1 month post-mastectomy. Data analysis is performed using mixed models and the tests are two-sided, with a type I error set at α=0.05.

Ethics and dissemination: The study protocol and the informed consent have been approved in December 2016 by the French Research Ethics Committee (South East VI Committee). Results will be communicated in different congress and published in international review.

Trial registration number: NCT03063931

Keywords

Magnesium, NMDA receptor, breast cancer, mastectomy, neuropathic Pain

Abbreviations

NP, Neuropathic Pain; NMDAR, *N*-Methyl-D-Aspartate receptor

Date and version identifier

January 2 2017, Version: 3

Sponsor

CHU de Clermont-Ferrand – 58, rue Montalembert, BP 69, F-63003 Clermont-Ferrand Cedex 1, France

INTRODUCTION

Breast cancer is the most common cancer in women worldwide and the lifetime probability of developing breast cancer is 12.3%, approximately 1 in 8.[1] Mastectomy, chemotherapy and radiotherapy play an important role in the development of neuropathic pain.[2] Neuropathic pain is defined as pain related to a lesion or disease affecting the somatosensory system. The mechanisms responsible for spinal hyperexcitability include the activation of central and peripheral *N*-methyl-D-aspartate receptor (NMDAR).[3,4] These play an ubiquitous role in pain central sensitization and in many other functions like memory and learning.[5-7] Neuropathic pain is associated with the development of a number of comorbidities including cognitive-emotional and sleep disorders.[8] Post-mastectomy pain may be reported in the anterior thorax, armpit, upper arm, and edema, sensory dysfunction, neuroma emergence, numbness in the arm contribute to the pain syndrome that affects 20 to 68% of the patients.[2] NMDAR antagonists such as ketamine, memantine, dextromethorphan or magnesium by blocking NMDAR may limit or even reverse the painful phenomena and are possible drugs for pain refractory to recommended treatments. [9-12]

With a translational approach, the prophylactic effect of memantine in neuropathic pain was recently demonstrated in animals and in humans.[12 13] In a preclinical pain model (Spinal Nerve Ligation) memantine has been shown to prevent neuropathic pain development when administered a few days before surgery. Molecular biology tests showed a decrease of pTyr^{1472}NR2B at spinal and supraspinal level (insula and hippocampus).[13] The translational clinical study confirmed the beneficial effect of memantine to prevent post-mastectomy pain development, diminish chemotherapy-induced pain symptoms and analgesic consumption, with a better quality of life for at least 6 months after surgery.[12]

Magnesium is a physiological NMDAR antagonist and blocks calcium and potassium channels of the receptor, modulating NMDAR activation with very few side-effects.[14]

Preclinical and clinical pain studies have reported the controverted curative effect of magnesium on pain with satisfactory[15-30] and mitigated results.[31-40] No study has so far focused the effect of several weeks oral administration of magnesium starting two weeks before surgery on post-operative pain and more specifically in post-operative pain related to breast cancer surgery.

In the present study, magnesium will be administered before surgery in order to evaluate its effect on pain development, cognition, emotion and quality of life during three months after mastectomy. The primary objective is to evaluate if magnesium administered two weeks before and 4 weeks after mastectomy may limit pain development at one month post-mastectomy compared to placebo. The secondary objectives are the evaluation of pain intensity, analgesic concomitant medications, cognitive-emotional function, quality of life and sleep one and three months after mastectomy

METHODS AND ANALYSIS

Study setting

A randomized, placebo-controlled, double-blind clinical trial will be conducted in the Oncology Hospital, Clermont-Ferrand, France, in 100 women undergoing total mastectomy for breast cancer. The study has been approved in December 2016 by the regional Ethics Committee and registered on February 24 2017 at "http://www.clinicaltrials.gov" (NCT03063931). Three weeks before surgery (D_{-21}), patients will meet the medical team and the physician will explain to the patient the protocol, the objectives of the study and the different questionnaires and tests that will be carried out in order for the patient to give her written informed consent. If necessary, a sufficient time for reflection will be granted. After having given informed consent, women will rate their pain on the numerical rating scale (NRS) and complete the cognition, emotion, quality of life and sleep questionnaires. A blood

test will be performed in order to dose the level of magnesium and creatinine, and participants will be randomized in two parallel groups: magnesium (n=50) or placebo (n=50). Patients will be contacted fifteen days before surgery in order to be reminded them to start their treatment. Magnesium or placebo (lactose) will be given orally for six weeks starting two weeks before surgery. Magnesium will be given at the dose of 100 mg/day (2 tablets of 50 mg/day once a day). Endpoints will be reassessed at one (M1) and three months (M3) post-mastectomy. Patients will be called once a week by phone in order to maintain a good compliance and to verify they do not develop adverse events. Finally, a booklet to monitor drug intake and adverse events will be completed daily by the patient for 3 months from the day of surgery (D_0). Detailed information on the present study is summarized in Figure 1.

Inclusion criteria

1. Patient is at least 18 years old.
2. Patient with a diagnosis of breast cancer and with planned total mastectomy with or without axillary dissection.
3. Patient with no change of treatment and diet.
4. Patient able to understand and agreeing to follow the study protocol.

Exclusion criteria

1. Patient with any magnesium contraindication: hypersensitivity to magnesium chloride or to any of the excipients.
2. Patient with a magnesemia of more than 1.05 mmol/L.
3. Patient with severe renal insufficiency and with a renal clearance of less than 30 mL/min.
4. Patient with an addiction to alcohol as diagnosed by the investigator.
5. Patient with diabetes (Type I and II).

6. Patient receiving treatment with quinidine or L-Dopa,
7. Patient in childbearing age, with no effective contraceptive method, pregnancy or lactation,
8. Patient enrolled in another clinical trial.
9. Patient with an inability to comply with the requirements of the protocol.

Intervention

Treatment group

The treatment group will receive magnesium during six weeks starting two weeks before surgery. Patients in the magnesium group should take once a day 100 mg/day ((2 x 50 mg) of low dose continuous release magnesium stored in opaque white bottles in order to maintain double blinding (CHRONOMAG®, FJ Life Sciences).

Control group

Patients will receive once a day two tablets of placebo (lactose) during six weeks starting two weeks before mastectomy.

Magnesium and packaging will be provided by FJ Life Sciences Society. Placebo will be prepared, conditioned and released in the Hospital Central pharmacy by one qualified person according to good manufacturing principles. The number of tablets in each dispensed container will be verified and recounted at the end of the treatment by two persons totally independent of the protocol.

Outcome evaluation

The primary endpoint will be the average pain intensity evaluation by NRS in magnesium and placebo groups over the 5 days before the one month post-surgery visit. The scale ranges from 0 no pain to 10 maximal tolerable pain.

The following secondary endpoints will be evaluated at the screening visit, M1 and M3: 1) pain evaluation with NRS and the McGill pain questionnaire;[41] 2) neuropathic pain questionnaire with the neuropathic pain in four questions (DN4) and the Neuropathic Pain Symptom Inventory questionnaire (NPSI);[42 43] 3) cognition with the Trail Making Test (TMT) and the Functional Assessment of Cancer Therapy-Cognitive Function (FACT-COG);[44 45] 4) anxiety and depression with the Depression Anxiety Stress Scale (DASS);[46] 5) quality of life with European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 items (EORTC QLQ-C30) and the Patient Global Impression of Change (PGIC) and 6) sleep with the Pittsburgh Sleep Quality Index (PSQI).[47-49] At the inclusion visit, M1 and M3, blood and urinary concentrations of magnesium will be measured. A summary of the evaluations for one patient is reported in table 1.

	STUDY PERIOD				
	Enrolment	Allocation	Post-allocation		Close-out
TIMEPOINT	**D0-21**	**D0-14**	**D0**	**M1**	**M3**
ENROLMENT:					
Eligibility screen	X				
Informed consent	X				
Allocation		X			
INTERVENTIONS:					
Start of treatment		X			
End of treatment				X	
Surgery			X		
Blood test	X			X	X
Urinary test				X	X
Delivery pain diary			X		
ASSESSMENTS:					
Numeric Rating Scale (NRS)	X			X	X
Neuropathic Pain four questions (DN4) **Neuropathic Pain Symptom Inventory (NPSI)**	X			X	X
McGill Pain questionnaire	X			X	X
Trail Making Test (TMT)	X			X	X
Functional Assessment of Cancer Therapy-Cognitive Function (FACT-COG)	X			X	X
EORTC QLQ-C30	X			X	X
Depression Anxiety Stress Scale (DASS)	X			X	X
Pittsburgh Sleep Quality Index (PSQI)	X			X	X
Patient Global Impression of Change (PGIC)	X			X	X
Concomitant analgesic treatments	X	X	X	X	X
Adverse events		X	X	X	X

- *McGill pain questionnaire*[41]

This questionnaire allows to qualify pain experience during the last 48 hours. It has fifty eight qualifiers divided into sixteen items (A to P). Each qualifier is rated from 0 to 4, where 0 = absent, 1 = low, 2 = moderate, 3 = strong, 4 = very strong. The score is divided between two subclasses, sensory subclass (items A to I) and emotional subclass (items J to P).

- *Neuropathic Pain in 4 questions questionnaire (DN4)*[42]

DN4 is a clinical tool for the diagnosis of neuropathic pain. This questionnaire has four questions divided into 10 items related to the interview (ie, symptoms) and to the sensory examination (ie, signs). The investigator asks and examines the patient and notes a response "no" or "yes" for each item: "yes" is scored as "1" and "no" is scored as "0". The sum of scores gives the total score of the patient (/10). DN4 is considered as positive if the patient obtains a score $\geq$ 4/10.

- *Neuropathic Pain Symptom Inventory (NPSI)*[43]

NPSI is a self-questionnaire and includes 10 pain descriptors. Intensity is rated on 0-10 numerical scales and two temporal items are designed to assess spontaneous ongoing pain duration and the number of pain paroxysms over 24h. This questionnaire discriminates 5 distinct clinically relevant dimensions: spontaneous burning pain, spontaneous deep pain, paroxysmal pain, evoked pain, and paresthesia/dysesthesia.

- *Trail Making Test (TMT)*[44]

This non-verbal cognitive test assesses the ability of speed, executive function, attention, concentration, visual perceptual speed. In Part A, circles are numbered from 1 to 25 and the patient must connect with lines the numbers in ascending order (1-2-3-4, etc.). In Part B, the circles contain numbers from 1 to 13 and letters from A to L, the patient must connect the circles with lines but alternating numbers and letters (1A-2B -3C, etc.). The patient must

connect the circles as quickly as possible for both parts of the test, without lifting the pen from the paper. The TMT B additionally provides an estimate of mental flexibility.

- *Functional Assessment of Cancer Therapy-Cognitive Function (FACT-COG)*[45]

This self-report questionnaire has been validated with cancer patients and assesses the impairment of cognitive ability and its impact on the patient's quality of life. It consists in 37 items assessing memory, attention, concentration, language and thinking abilities. The items are rated using a 5-point Likert scale. The FACT-COG takes into consideration the functional implication of cognitive impairment, the deficits observed by other people, the changes in cognitive function over time, and their impact on the patient's quality of life.

- *Depression Anxiety Stress Scale*[46]

The DASS is a 42-item self-report instrument designed to measure the three related negative emotional states of depression, anxiety and tension/stress. The DASS Depression focuses on reports of low mood, motivation, and self-esteem, DASS-anxiety on physiological arousal, perceived panic, and fear, and DASS-stress on tension and irritability. A respondent indicates on a 4-point scale the extent to which each of 42 statements applied over the past week. A printed overlay is used to obtain total scores for each subscale. Higher scores on each subscale indicate increasing severity of depression, anxiety or stress.

- *European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 items (EORTC QLQ-C30)*[47]

This questionnaire assesses the quality of life of cancer patients. It is divided in 9 subscales consisting of several items: 5 subscales measuring functional status (physical, role, social, emotional, cognitive), three subscales measuring symptoms (fatigue, pain, nausea and vomiting) and a global subscale of quality of life and health. Finally, six items/isolated symptoms, covering cancer symptoms and frequent side effects of cancer therapies are also included in the EORTC QLQ-C30.

- *Patient Global Impression of Change*[48]

This is a 7-point self-reported numerical scale used to assess what the change in their condition following treatment meant to the patient.

- *Pittsburgh Sleep Quality Index*[49]

This questionnaire consists of 19 items and is used to measure sleep quality. It consists of 7 domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication and daytime dysfunction.

Recruitment and Randomization

Three weeks before mastectomy, when the informed consent is signed, blood samples (magnesium and creatinine concentrations) and questionnaires will be performed. Then, patients will be randomized in the magnesium (n=50) or placebo group (n=50). Treatment allocation will follow a predetermined randomization list and will be carried out by a person totally independent from the protocol. The randomization sequence will be generated using random blocks. Treatments will be packed in a similar opaque bottle covered with an identical label indicating batch number, expiry date and sponsor code with no indication of the name of the drug. In order to maintain blinding, the physician who evaluated pain could not guess allocation at any time and would not meet the patient again in the course of the trial.

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.[50] Considering the literature, the prevalence of post-mastectomy pain is 20%. [51, 52] However, these data may vary depending on demographic, psychological and medical/surgical factors [53] and will be taken into consideration in this study. 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 NRS as a covariate will be proposed as multivariable analysis, as proposed by Vickers and Altman (Vickers and Altman, 2001).[54] 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".[55]

Data handling and record keeping

All original records such as consent forms, Case Report Forms, questionnaires and pain diary will be archived at trial sites for 15 years. The database file will be anonymized and maintained also for 15 years. The monitoring will be performed by a clinical research associated independent from the protocol. Then, the monitored case report forms will be transferred to the Data Management Center (CIC-Inserm 1405, Clermont-Ferrand).

Duration of the study

The duration of treatment will be 42 days. The total duration of participation per patient will be of 14 weeks. The protocol will include 4 visits (D_{-21}/D_0/M1/M3) including a period of hospitalization per patient. Treatment will be given daily starting two weeks before surgery and maintained four weeks after. The recruitment will start in June 2017. The total duration of the study is estimated at two years.

Ethics and dissemination

The study received approval by the French Research Ethics Committee on December 2016 (ID-RCB n° 2016-A01749-42). The trial is registered in ClinicalTrials.gov (trial n° NCT03063931). Each patient meeting the inclusion criteria will sign a Consent Form after receiving oral and written information. After agreement between all investigators, data will be disclosed and results will be communicated in different congress and published in international review.

DISCUSSION

Following successful results obtained with prophylactic memantine in neuropathic pain development,[12 13] this study aims at assessing magnesium treatment in a similar protocol

to prevent neuropathic pain induced by mastectomy. In breast cancer surgery, clinical studies using magnesium have focused so far on the qualitative and emotional aspects of pain rather than on the intensity of pain itself.[56] Magnesium in neuropathic pain alleviation has shown controverted results.[19-40]Magnesium has been shown to modulate the limbic system *via* NMDAR and these brain areas are known to be involved in emotion and pain.[57] It is therefore essential to evaluate concomitantly magnesium effect on pain and also on cognitive-emotional and sleep aspects. Magnesium deprivation may affect cognition and sleep quality. Preclinical findings showed that an increase in brain magnesium enhances both short-term synaptic facilitation and long-term potentiation and improves learning and memory functions.[58] In human, a study showed that preeclamptics patient receiving magnesium had better attention and working memory performance both before and after delivery compared to controls.[59] Furthermore, a review reported the relationship between low level of magnesium, stress and cognitive difficulties such as lack of concentration and difficulties in learning.[60] Concerning the impact of magnesium on sleep, a placebo-controlled, randomized cross-over study performed in 12 older participants showed that magnesium supplementation significantly reversed electroencephalogram changes, including decreased slow wave sleep, that may occur during aging.[61] Furthermore, a double blind trial reported that intraoperative infusion of magnesium led to a significantly better quality of sleep during the post-operative period without any side-effects.[62]

Magnesium is an abundant mineral, naturally present in food and is available as a dietary supplement that is appreciated by patients.[63] It is obtained without prescription and has a favourable risk-benefit balance with few side effects.[63] This molecule is also known to regulate diverse biochemical reactions in the body and is required for energy production, oxidative phosphorylation, and glycolysis. It also plays a role in the active transport of calcium and potassium ions across cell membranes, a process that is important to nerve

impulse conduction, muscle contraction, and normal heart rhythm.[64 65] Low blood levels of magnesium have been associated with a number of pathologies including type-2 diabetes, or cardio-vascular disease.[66] Oral magnesium supplementation is usually well tolerated and gastrointestinal side effects including nausea, vomiting, and diarrhea are usually minor.[66 67]

The pharmaceutical form in this trial provides magnesium chloride, a circulating form of magnesium with a gradual and constant release of low doses of magnesium along the gastro intestinal tract. A recent clinical study (NCT01935570) showed that the dose of 100 mg daily guarantees an optimal absorption of magnesium by the body over a 24-hour period. Furthermore, this form of magnesium does not induce intestinal side effects and is easy to use with a once a day intake.

In conclusion, if magnesium given before and after mastectomy proves its efficacity in neuropathic pain prevention, it could be an excellent prophylactic strategy to prevent post-mastectomy pain symptoms, maintain quality of life and cognitive function and limit comorbidities that accompany breast cancer pathology.

Authors' contributions

GP is the overall study principal investigator; she participated in the conception and study design and contributed to the writing of the study protocol and the drafting and editing of this manuscript. DJ, CV and BP all participated in the study design. BP contributed to the writing of the study protocol and carried out all statistical calculations and wrote the statistical paragraph in the study protocol. He contributed with GP and VM to the drafting and editing of this manuscript. All authors read and approved the final manuscript.

Funding

University Hospital Clermont-Ferrand, France

Competing interests

The authors declare that there are no financial or non-financial competing interests neither within the conception nor conduction of the trial.

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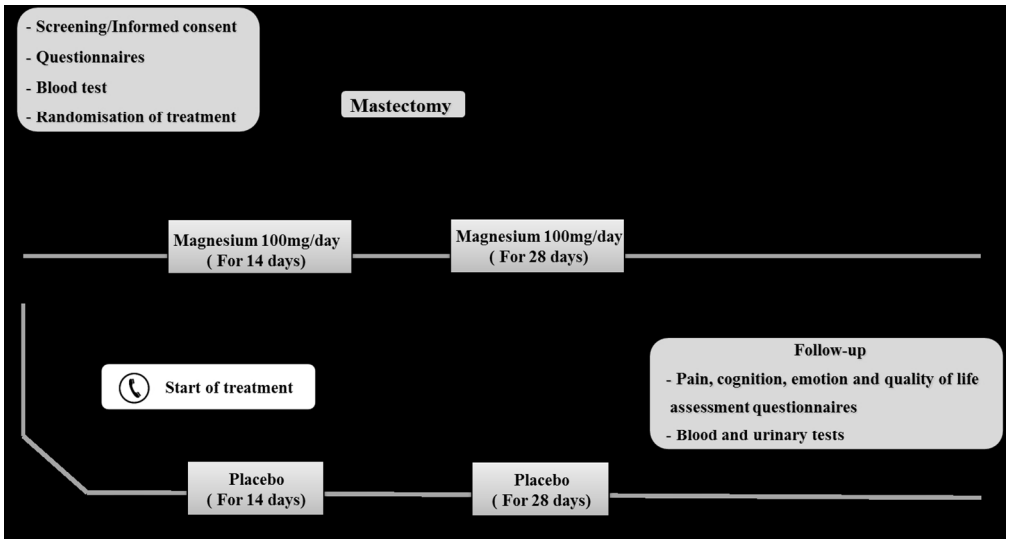
Figure legends

Figure 1: Study design

Table legend

Table 1: Summary of assessments



Study design

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____2_____
	2b	All items from the World Health Organization Trial Registration Data Set	_____NA____
Protocol version	3	Date and version identifier	_____3_____
Funding	4	Sources and types of financial, material, and other support	_____15_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	__1 and 3_____
	5b	Name and contact information for the trial sponsor	_____3_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____/_____
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____15_____

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	/
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5-6
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	/
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	_11-12_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	____11_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	__11_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	__11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	__11_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	__11_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____/_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	___5-6_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___5-6_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_11_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_12_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_12_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_12_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_13_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____/_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____/_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____/_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	__13_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____/_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___13_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	_____/_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	___13_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	___15_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	___13_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____/_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	___13_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____/_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_____/_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_____/_____
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_____/_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.