

Research Article

The anxiolytic and analgesic effects of melatonin: a study protocol for a randomized, double-blind, placebo-controlled study

Benedikte Kollerup Madsen^{1*}, Dennis Zetner¹, Lars Peter Kloster Andersen², Jacob Rosenberg¹

¹Department of Surgery D, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark

²Department of Anesthesia and Intensive Care Z, Bispebjerg and Frederiksberg Hospital, University of Copenhagen, DK-2400 Copenhagen, Denmark

*Correspondence: Benedikte.kollerupmadsen@gmail.com; Tel: +45 28188624.

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ABSTRACT

Anxiety and pain are both significant clinical challenges following surgery. Melatonin has been demonstrated to have both anxiolytic and analgesic effects in numerous experimental and clinical studies. This study protocol aims to present the design of a randomized study investigating the anxiolytic and analgesic effects of exogenous melatonin in cosmetic breast augmentation.

The study will be a randomized, double-blind, placebo-controlled trial. Included patients are candidates for primary breast augmentation or replacement of existing implants. Patients receive a standardized general anesthetic and postoperative analgesic regimen. The study medication includes 4 separate doses, containing either 10 mg of melatonin or placebo. Doses are administered orally at 21:00 hours of the night before surgery, 120 minutes before surgery, immediately after surgery in the post-anesthesia care unit, and at 21:00 hours of the night following surgery.

The two primary outcomes are preoperative anxiety, assessed by the State-Trait Anxiety Inventory and pain, measured by an integrated assessment of longitudinally measured pain intensity and opioid consumption. Secondary outcomes are VAS anxiety, and VAS pain, sleep quality, general fatigue and wellbeing.

This study protocol presents in detail the design of a randomized, double-blind, placebo-controlled study investigating the anxiolytic and analgesic effects of repeated doses of exogenous melatonin in patients undergoing cosmetic breast augmentation.

Key words: melatonin, anxiety, pain, perioperative.

Trial registration: Clinicaltrials.gov: NCT02386319. Capital Region's Committee on Health Research Ethics protocol number: H-8-2014-016. The Danish Data Protection Agency journal number: VD-2019-101. The Danish Medicines Agency EudraCT number: 2014-003789-25.

1. INTRODUCTION

Anxiety and pain are potential clinical challenges in the perioperative period. Perioperative anxiety influences postoperative pain, increases analgesic requirements, and delays recovery (1), whereas postoperative pain may induce sleep disturbances and reduce patient satisfaction (2, 3). Breast

augmentation is the most common cosmetic surgical procedure in the United States (4), however, few studies have investigated optimal analgesic regimens in this patient category (2).

Recent studies have shown that widely used analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs) and opioids may cause serious adverse effects and increase the risk of postoperative complications (5, 6). Also, anxiolytics such as benzodiazepines can cause a variety of adverse effects (7). Therefore, continuous investigation of alternative anxiolytics and analgesics is needed to optimize multimodal anxiolytic and analgesic regimens.

Melatonin regulates sleep and circadian rhythm in mammals (8). Furthermore, numerous animal studies have demonstrated dose-dependent anxiolytic and analgesic effects of exogenous melatonin (9, 10), and clinical studies in humans have documented consistent anxiolytic and analgesic effects of melatonin in surgical patients (11).

In this study protocol, we present the design of a randomized, double-blind, placebo-controlled study investigating the anxiolytic and analgesic effects of exogenous melatonin in cosmetic breast augmentation.

2. METHODS

2.1. Study design and setting.

The study is a randomized, double-blind, placebo-controlled clinical trial. Patients will be randomized to receive either melatonin or placebo. The study will be reported in accordance to CONSORT guidelines (12). The study will be performed at one or more hospitals in the Capital Region of Denmark.

2.2. Eligibility criteria.

Patients will be included in the study according to predefined eligibility criteria (see Table 1).

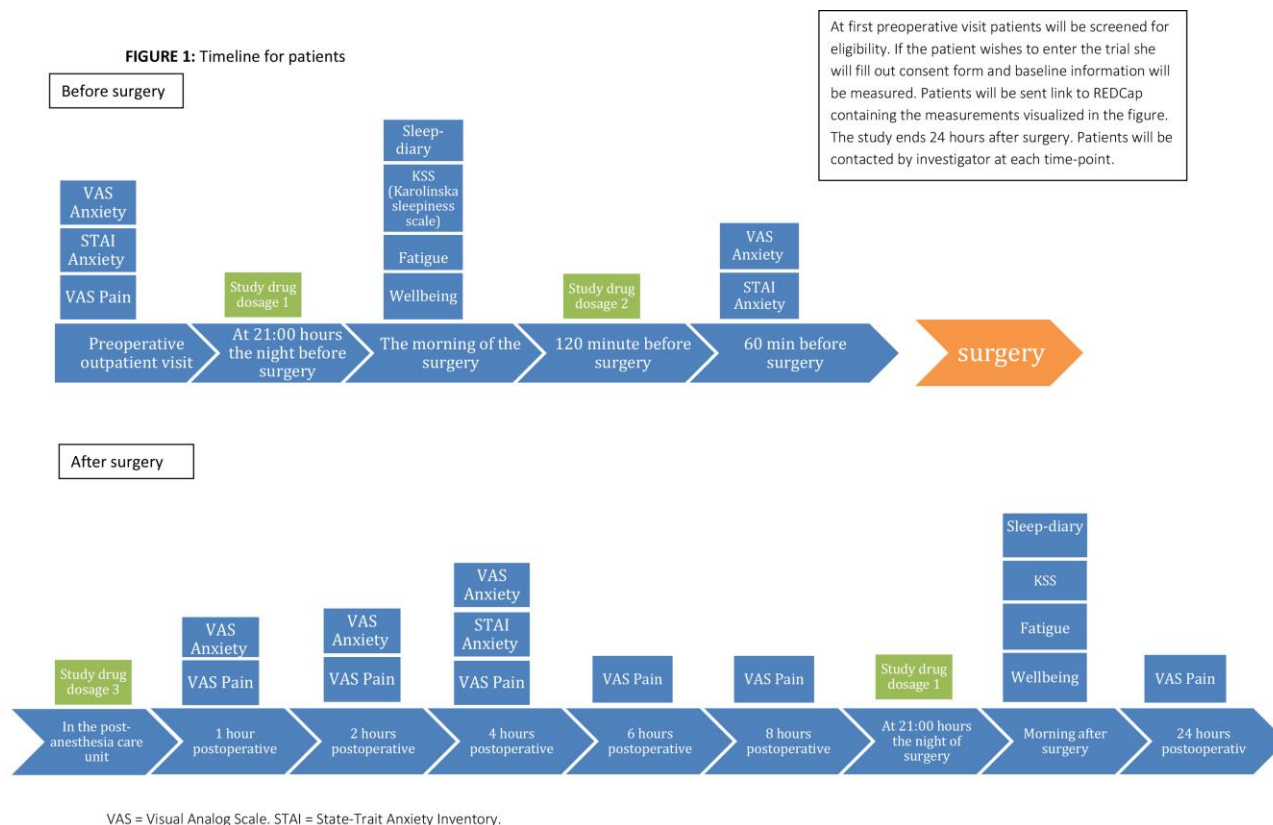
Table 1: Inclusion and exclusion criteria.

INCLUSION CRITERIA	EXCLUSION CRITERIA	WITHDRAWAL AND DROP-OUT-CRITERIA
<ul style="list-style-type: none"> • Women • Candidates for primary breast augmentation or secondary replacement of existing implants 	<ul style="list-style-type: none"> • Daily use of opioids, benzodiazepines or melatonin • Psychiatric illness (defined as a medically treated disorder) • Physical disease (defined by ASA physical status classification system > II) • Current or former alcohol abuse (defined as >168 g per week) or current drug abuse (defined as current use of an illegal drug) • Liver disease (defined as a medically treated disorder) • Known sleep disorder (defined as a medically treated disorder) • Know allergy to melatonin or melatonin-products • Unable to cooperate with the study protocol 	<ul style="list-style-type: none"> • Patients can withdraw at any time during trial • Complications during surgery leading to: <ol style="list-style-type: none"> 1. Reoperation within the first 24 postoperative hours 2. Hospitalization to intensive care unit • Positive pregnancy-test (performed before 21:00 hours the night before surgery) • If it is considered in the best interest of the patient's physical and psychological health to withdraw from the trial

ASA=American Society of Anesthesiologists Score

2.3. Patient inclusion and follow-up.

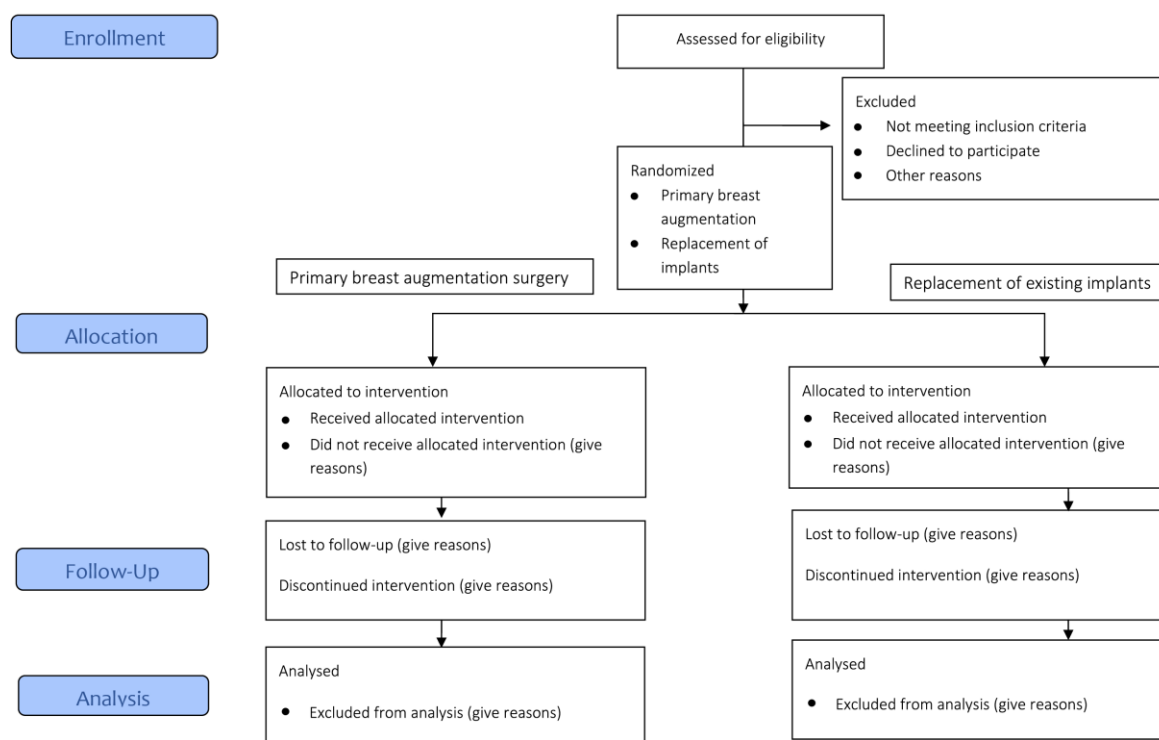
A patient timeline is illustrated in Figure 1. Patients will be informed about the study by the investigator at the first preoperative outpatient visit. The patients will be informed that they are entitled to 24 hours of consideration before accepting or declining to enter the trial. If patients agree to enter the trial and meet the eligibility criteria, verbal and written consent will be obtained. Patients will be monitored the first 24 hours after surgery.



2.4. Randomization and blinding.

Randomization and packaging of study medication is performed by an independent Good Manufacturing Practice (GMP)-approved pharmacy (Skanderborg Apotek, Skanderborg, Denmark). The randomization allocation list is generated by a computer-based algorithm. Patients will be randomized following the inclusion procedure (Figure 2). The investigator provides the study medication in sealed envelopes according to the randomization list. Patients will be stratified according to the type of surgery: primary breast augmentation or replacement of existing implants. Block-randomization (each block containing 4 patients) will be performed until at least 36 patients in each treatment group have completed the study. Since patients are stratified according to surgical procedure, a new block receiving same surgical procedure as the drop out must be included, if a patient drops out. Patients and study personnel are blinded to treatment allocation until end of trial. Investigators performing data analyses are also blinded. If suspected unexpected serious adverse reactions (SUSARs) occur, the primary investigator will evaluate if unblinding of patient treatment allocation is necessary.

FIGURE 2: CONSORT Flow Diagram



2.5. Intervention.

Both active medication and the placebo consist of gelatine capsules. The active medication contains 10 mg of melatonin. Active medication and placebo are identical in colour, shape and taste. Four separate doses are administered orally at 21:00 hours the night before surgery, 120 minutes before surgery, immediately after surgery in the post-anesthesia care unit, and at 21:00 hours on the night of surgery. Patients will be reminded to take study medication by study personnel, either in person or by phone-call/text-message/e-mail.

2.6. Surgery.

Surgical procedures include either primary breast augmentation or replacement of existing implants. All procedures are by subpectoral technique. Patients are admitted 90 minutes before surgery, except for surgical procedures commencing at 08:00 hours in which case patients are admitted 45 minutes prior to procedure. All surgical procedures are performed between 08:00 hours and 16:00 hours. Patients receive a standardized general anaesthetic and postoperative analgesic regimen (Table 2).

TABLE 2: Description of standard anesthetic and analgesic regimens.

PREMEDICATION
<ul style="list-style-type: none"> • Acetaminophen 1 g • Dexamethasone 8 mg • Etodolac 300 mg
INTRAOPERATIVE
<p><i>Anesthetics, induction</i></p> <ul style="list-style-type: none"> • Propofol 2-3 mg/kg <p><i>Anesthetics, maintenance</i></p> <ul style="list-style-type: none"> • Remifentanyl 0.5 µg/kg/min • Propofol 3-4 mg/kg/hr • Sufentanyl 0,3 ug/kg, 10 min before completion of surgery <p><i>Local anesthetics</i></p> <ul style="list-style-type: none"> • Bupivacaine 0.5% with adrenaline 0.6 ml/kg sc
POSTOPERATIVE
<ul style="list-style-type: none"> • Oxycodone 5 mg iv, as needed (in the PACU) • Acetaminophen 1 g x 4 • Ibuprofen 400 mg x 4 • Chlorzoxazon 250 mg x 3 • Oxycodone 5 mg, as needed

PACU= Post-anesthesia care unit.

3. OUTCOMES

3.1 Primary outcomes.

1. Preoperative anxiety 60 minutes before surgery, assessed by the State-Trait Anxiety Inventory (STAI) (13).

The STAI contains a total of 40 questions (13). Twenty questions relate to state anxiety by estimating the immediate level of anxiety and other twenty questions relate to trait anxiety, as a measure of a person's general level of fearfulness (13). The trait anxiety will only be assessed at the preoperative visit. The questionnaire has not previously been validated to Danish language. Hence, the study will include a validation of the Danish edition of STAI.

2. Postoperative pain, measured by an integrated assessment of longitudinally measured pain intensity and opioid consumption (PIOC) (0-24 hours) (14).

The integrated assessment combines Area Under Curve (AUC) estimates of multiple Visual Analogue Scale measurements of pain (VAS pain) and opioid consumption into a single outcome. Individual VAS scores (0 mm = no pain, 100 mm = worst imaginable pain) will be assessed at 1, 2,

4, 6, 8, and 24 hours postoperatively. Scores will be performed during movement from supine to upright position.

3.2. Secondary outcomes.

Preoperative anxiety will also be assessed by a VAS (0 mm = no anxiety, 100 mm = worst imaginable anxiety). In addition, postoperative anxiety will be assessed by additional STAI and VAS measurements (Figure 1), PIOC scores at rest (0-6 hours/0-24 hours) and during movement (0-6 hours) will also be calculated. Also, intraoperative use of propofol and remifentanyl will be recorded.

Postoperative sleep quality will be measured by subjective sleep quality assessments using a VAS (0 mm = worst imaginable sleep, 100 mm = best imaginable sleep). A sleep diary describing total sleep time, and number and length of awakenings will also be performed. The Karolinska Sleepiness Scale will be applied to assess subjective sleepiness (15). The scale includes a 9-point interval scale (1 = extremely alert, 9 = extremely sleepy – fighting sleep). Fatigue will be assessed on a 10-point interval scale (1 = fresh, 10 = extremely tired) (16). General wellbeing will be monitored with a VAS scale (0 mm = very fine, 100 mm = extremely uncomfortable).

4. DATA PROCESS

4.1. Data management.

Research Electronic Data Capture (REDCap) will comprise individual case report forms. Patients will receive a link to REDCap at standardized timepoints (Figure 1). In REDCap, patients will be able to register STAI-questionnaire results, VAS anxiety/pain measurements, sleep-diary questionnaires and analgesic consumption. Baseline characteristics, intraoperative data and occurrence of adverse reactions will be provided to REDCap by study personnel. The Good Clinical Practice (GCP)-Unit, the Danish Medicines Agency and delegated investigators will be able to access data. Data will be kept for 10 years before being erased.

4.2. Sample size calculations.

We intend to report data of each primary outcome in two separate publications. Therefore, individual sample size calculations for each primary outcome is calculated separately.

No previous studies have assessed preoperative anxiety in relation to cosmetic breast augmentation. Therefore, the sample size is based on a previous study measuring preoperative anxiety applying STAI in patients undergoing various elective surgical procedures (17). Preoperative anxiety, specified as State-anxiety 1 hour before surgery, documented a mean (SD) value of 39.30 (9.30) (17). We defined a statistical power of 80%, a significance level of 5% and a minimal relevant difference (MIREDF) of 30%. From these assumptions, 10 patients in each treatment group are needed.

Correspondingly, no previous studies have applied the integrated assessment in cosmetic breast augmentation. Hence, a sample size calculation is based on VAS pain 6 hours following breast augmentation. A previous study has demonstrated a mean VAS (SD) pain of 4.6 (2.1) (18). We applied a power of 80%, a significance level of 5%, and a MIREDF of 30%. From these assumptions, 36 patients in each treatment group are needed.

We will therefore include a total of at least 36 patients in each group, where 10 patients in each group should have complete data for preoperative STAI assessment, and 36 patients have complete data for PIOC covering 0-24 hours postoperatively.

4.3. Statistical analyses.

Difference in anxiety between the treatment groups, assessed by STAI (13) 60 minutes prior to surgery (melatonin and placebo) will be calculated using Mann-Whitney test or unpaired t-test, depending on the distribution of data.

Postoperative pain will be assessed by the integrated assessment PIOC (14). Individual PIOC scores are calculated by ranking each patient according to the AUC VAS pain and opioid consumption separately (19). A mean rank of all patients (active treatment + placebo) will be calculated. The difference between mean rank and the individual patient's rank is expressed as percentage of the mean rank (% difference). The two % differences (AUC VAS pain/opioid consumption) are summed. The summed % difference represents the integrated score of each patient (range -200 to +200%). A summed % difference value above zero indicates increased "pain". Individual scores will be compared between treatment groups using Mann-Whitney test or unpaired t-test, based on the distribution of data.

Multiple linear regression analysis will be used to assess which variable affects preoperative anxiety and postoperative pain (sleep, implant size, trait-anxiety at baseline, and/or age). Other measuring points and individual statistical tests for anxiety, pain, sleep quality, fatigue, and wellbeing are described in detail in Table 3. A p-value < 5% is considered statistically significant. Bonferroni correction of the primary outcomes is not viewed as relevant. Secondary outcomes are exploratory and will not be corrected. Assessments of normality will be performed by visual inspection of histograms/residual plots. Data will be reported as mean (SD) or median (IQR or range) depending on distribution. Data will be analysed using IBM SPSS Statistics for Windows version 25.0 (IBM Corp, Armonk, NY, USA).

TABLE 3: Outcomes, individual measuring points, and statistical plan.

OUTCOME	MEASURING POINT(S)	STATISTICAL TEST
Preoperative outpatient visit		
Age (years)	<ul style="list-style-type: none"> At the preoperative visit 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
BMI (kg/m²)	<ul style="list-style-type: none"> At the preoperative visit 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
VAS pain (mm)	<ul style="list-style-type: none"> At the preoperative visit 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
Anxiety (STAI)	<ul style="list-style-type: none"> At the preoperative visit 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
VAS anxiety (mm)	<ul style="list-style-type: none"> At the preoperative visit 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data

Size of breast implant (mL)	<ul style="list-style-type: none"> At the preoperative visit 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
Surgical course		
Pre- and postoperative anxiety (STAI)	<ul style="list-style-type: none"> 60 minutes before surgery 4 postoperative hours 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> Intragroup comparison of repeated STAI anxiety measures Intergroup comparison of repeated STAI anxiety measures 	Repeated measures ANOVA
	<ul style="list-style-type: none"> Difference in anxiety (baseline – 60 minutes preoperatively) in placebo- and active group. Difference in anxiety (60 minutes preoperative – 4 hours postoperatively) in placebo- and active group. 	Wilcoxon's signed rank test or paired two sample T-test, depending on distribution of data
Integrated pain score during movement (%)	<ul style="list-style-type: none"> Composite outcome of AUC of movement-related pain scores at the PACU and at 1, 2, 4, 6, 8 and 24 postoperative hours and opioid requirements (0-24 postoperative hours) 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> Intragroup comparison of repeated VAS pain measurements Intergroup comparison of repeated VAS pain measurements 	Repeated measures ANOVA
Integrated pain score during rest (%)	<ul style="list-style-type: none"> Composite outcome of AUC during rest pain scores at 1, 2, 4, 6, 8 and 24 postoperative hours and opioid requirements (0-24 postoperative hours) 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> Intragroup comparison of repeated VAS pain measurements 	Repeated measures ANOVA

	<ul style="list-style-type: none"> • Intergroup comparison of repeated VAS pain measurements 	
Pre- and postoperative VAS anxiety (mm)	<ul style="list-style-type: none"> • 60 minutes before surgery • In the post anesthesia care unit • 1, 2 and 4 postoperative hours 	Intergroup comparisons by singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> • Intragroup comparison of repeated VAS anxiety measurements • Intergroup comparison of repeated VAS anxiety measurements 	Repeated measures ANOVA
	<ul style="list-style-type: none"> • Difference in anxiety (baseline – 60 minutes preoperatively) in placebo- and active group. • Difference in anxiety (60 minutes preoperatively – 4 hours postoperatively) in placebo- and active group. 	Wilcoxon's signed rank test or paired two sample T-test, depending on distribution of data
Intraoperative requirements of remifentanil (µg), propofol (mg) and sufentanil (µg)	<ul style="list-style-type: none"> • At surgical completion 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
Opioid requirements (0-24) (mg)	<ul style="list-style-type: none"> • After 24 postoperative hours 	Unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
VAS sleep (mm)	<ul style="list-style-type: none"> • The morning before surgery • The morning after surgery 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> • Difference in sleep (preoperative - postoperative) 	Wilcoxon's signed rank test or paired two sample T-test, depending on distribution of data
Total sleep-time (min)	<ul style="list-style-type: none"> • The morning before surgery • The morning after surgery 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> • Difference in sleep time (preoperative - postoperative) 	Wilcoxon's signed rank test or paired two sample T-test,

		depending on distribution of data
Number of awakenings (n)	<ul style="list-style-type: none"> The morning before surgery The morning after surgery 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> Difference in number of awakenings (preoperative – postoperative) 	Wilcoxon's signed rank test or paired two sample T-test, depending on distribution of data
Length of awakenings (min)	<ul style="list-style-type: none"> The morning before surgery The morning after surgery 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> Difference in length of awakenings (preoperative – postoperative) 	Wilcoxon's signed rank test or paired two sample T-test, depending on distribution of data
Karolinska sleepiness scale (points)	<ul style="list-style-type: none"> The morning before surgery The morning after surgery 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
	<ul style="list-style-type: none"> Difference in Karolinska sleepiness scale (preoperative – postoperative) 	Wilcoxon's signed rank test or paired two sample T-test, depending on distribution of data
VAS general wellbeing (mm) and fatigue scale (points)	<ul style="list-style-type: none"> The morning before surgery The morning after surgery 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
Time spent in the post-anesthesia care unit (PACU) (hours)	<ul style="list-style-type: none"> Measured from admission to PACU and to patient is discharged 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data
Total time hospitalized (hours)	<ul style="list-style-type: none"> Measured from end of surgery to patient is discharged 	Singular unpaired two-samples T-test or Mann-Whitney, depending on distribution of data

BMI = Body Mass Index. VAS = Visual Analog Scale. STAI = State Trait Anxiety Index. ANOVA = Analysis of variance.

5. OTHERS

5.1. Harms.

Numerous studies have documented that melatonin is a non-toxic molecule (20). Its adverse effects may include light dizziness, mild headache, mild nausea and light drowsiness (20) and this

will be registered if spontaneously reported by patients. Complications that can be attributed to the surgery will not be registered as adverse events in this trial. Patients are informed that they can contact investigator at any time and report adverse events. Due to the non-toxic nature of melatonin, a follow-up of 24 hours following surgery is considered sufficient. Serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) will be reported to relevant authorities according to Danish law.

5.2. Registrations and approvals.

The study has been registered at clinicaltrials.gov (Clinicaltrials.gov identifier: NCT02386319) as well as in the European Clinical Trials Database (EudraCT) (registration: 2014-003789-25). Approvals from the Capital Region's Committee on Health Research Ethics (Protocol number: H-8-2014-016), the Danish Data Protection Agency (Journal number: VD-2019-101) and the Danish Medicines Agency have been granted. The study will be performed in accordance to the Helsinki II declaration. The study will be monitored by the GCP-unit, Copenhagen University Hospital.

5.3 Dissemination policy.

The data of the study will be presented in two separate publications. The first publication reports the anxiolytic and sleep-regulating effect of melatonin. This publication will also include a language validation of the STAI (13). The second publication reports analgesic effects. Authors are required to adhere to ICMJE authorship criteria (21).

6. DISCUSSION

This study protocol presents the design of a randomized, double-blind, placebo-controlled study investigating the anxiolytic and analgesic effects of exogenous melatonin in cosmetic breast augmentation.

Previous meta-analyses demonstrated that exogenous melatonin significantly reduced *preoperative* VAS anxiety by 19 mm, and *postoperative* VAS pain by 20 mm in a wide range of surgical procedures (22). However, the included studies were heterogeneous, and contained a significant risk of bias. Therefore, further investigations of melatonin are needed, due to the safe nature of the compound, and the vast number of experimental studies documenting both anxiolytic and analgesic properties. In our opinion, cosmetic breast surgery provides an ideal clinical pain model since patients lack serious comorbidity, and typically suffer from clinically relevant postoperative pain (18). Furthermore, this study includes validated questionnaires of anxiety, and detailed assessments of longitudinal-measured movement-related pain. We chose to include repeated administrations of melatonin to ensure sufficient plasma-levels during the entire perioperative period. Also, we chose to administer 10 mg melatonin, which corresponds to previous studies demonstrating anxiolytic and analgesic effects of melatonin in the perioperative period (11). Finally, we included detailed sleep questionnaires, which may elucidate any possible positive sleep-regulating effects of melatonin in surgical patients.

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AUTHORSHIP

All authors made substantial contributions to the design of the study. The manuscript was mainly drafted by LPA and BKM, and critically reviewed by DZ and JR. All authors read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors claimed no conflict of interest.

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