The effect of MELatOnin on Depression, anxietY, cognitive function and sleep disturbances in patients with breast cancer. The MELODY trial: protocol for a randomized, placebo-controlled, double-blinded trial.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000647
Article Type:	Protocol
Date Submitted by the Author:	18-Nov-2011
Complete List of Authors:	Hansen, Melissa; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section Madsen, Michael; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section Hageman, Ida; Rigshospitalet, University of Copenhagen, Psychiatric Center Copenhagen Rasmussen, Lars; Rigshospitalet, University of Copenhagen, Department of Anaesthesia, Centre of Head and Orthopaedics Bokmand, Susanne; Herlev Hospital, University of Copenhagen, Department of Breast Surgery Rosenberg, Jacob; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section Gögenur, Ismail; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Oncology, Pharmacology & therapeutics
Keywords:	Breast surgery < SURGERY, Depression & mood disorders < PSYCHIATRY, Delirium & cognitive disorders < PSYCHIATRY, SLEEP MEDICINE, Anxiety disorders < PSYCHIATRY, Clinical trials < THERAPEUTICS

Title page – study protocol

The effect of MELatOnin on Depression, anxietY, cognitive function and sleep disturbances in patients with breast cancer. The MELODY trial: protocol for a randomized, placebo-controlled, double-blinded trial.

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Key words: breast cancer, melatonin, depression, sleep disturbances, cognitive function

Word count: 2959

ABSTRACT

Introduction: Breast cancer represents about one third of all cancer diagnoses and accounts for about 15 % of cancer deaths in women. Many of these patients experience depression, anxiety, sleep disturbances and cognitive dysfunction. This may adversely affect quality of life but also contribute to morbidity and mortality. Melatonin is a regulatory circadian hormone having, among others, a hypnotic and an anti-depressive effect. It has very low toxicity and very few adverse effects compared with the more commonly used antidepressants and hypnotics.

Methods and analysis: The objective of this double-blind randomized, placebo-controlled trial is to investigate whether treatment with oral melatonin has a prophylactic or ameliorating effect on depressive symptoms, anxiety, sleep disturbances and cognitive dysfunction in women with breast cancer. Furthermore we will examine whether a specific clock-gene PER3 is correlated with an increased risk of depressive symptoms, sleep disturbances or cognitive dysfunction.

The MELODY-trial is a prospective double-blinded, randomized, placebo-controlled trial in which we intend to include 260 patients. The primary outcome is depressive symptoms measured by the Major Depression Inventory (MDI). The secondary outcomes are anxiety measured by a visual analogue scale (VAS), minutes of sleep, sleep effectiveness, sleep latency and periods awake measured by actigraphy and changes in cognitive function measured by a neuropsychological test-battery. Tertiary outcomes are fatigue, pain, well-being and sleep quality/quantity measured by VAS, sleep diary and sleepiness measured by the Karolinska Sleepiness Scale. The PER3 genotype is also to be determined in blood samples.

Ethics: The study is approved by the local ethics committee (H-4-2011-007). The study has also been approved by The Danish Medicines Agency (EudraCT nr. 2010-022460-12) and The Danish Data Protection Agency (2007-58-0015/HEH.750.89-12). The project is registered on www.clinicaltrials.gov - clinicaltrials.gov identifier: NCT01355523.

INTRODUCTION

Breast cancer is the most common type of cancer among women worldwide with about 1.4 million new cases every year [1]. Breast cancer accounts for 31 % of the various cancer diagnoses in women and is responsible for 15 % of deaths in females due to cancer [2]. For Danish women it is the most common type of cancer and the incidence is rising [3]. The incidence rises towards the age of 65 and then the curve flattens out [3]. Improvement of treatment has led to increasing 5 year-survival which is now 84 % in Denmark [4] and 89 % in the US [5]. Now focus has turned to optimizing quality of life because these patients may have a number of different psychological and physical symptoms such as depression, anxiety, fatigue, cognitive dysfunction and sleep disturbances [6-12].

METHODS

Study design and objective

MELODY (The effect of **MEL**at**O**nin on **D**epression, anxiet**Y**, cognitive function and sleep disturbances in breast cancer patients) is a prospective double-blinded, randomized, placebo-controlled trial in which we intend to include 260 patients undergoing surgery for breast cancer. The objective is to investigate whether treatment with oral melatonin has a prophylactic or ameliorating effect on depressive symptoms, anxiety, sleep disturbances and cognitive dysfunction in women with breast cancer. Furthermore, we will examine whether a specific clock-gene PER3 is correlated with an increased risk of depressive symptoms, sleep disturbances or cognitive function. Patients in the intervention group will receive 6 mg oral melatonin daily 1 hour before bedtime for 1 week preoperatively to 12 weeks postoperatively. Patients in the control group will receive placebo.

Study population:

Table 1: Inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
• Female between 30-75 years • Lumpectomy or mastectomy • American Society of Anesthesiologists (ASA) class I-III • No sign of depression on Major Depression Inventory (MDI) • Not pregnant	• Neoadjuvant chemotherapy • Treatment with: - Selective serotonin reuptake inhibitors (SSRI) - Antithrombotic drug therapy (except 75 mg acetylsalicylic acid (ASA) daily) - Monoaminoxidase (MAO) inhibitors - Calcium channel blockers • Rotor or Dubin-Johnson syndrome • Epilepsy • Known allergic reaction to melatonin • Known and treated sleep apnea

	• Diabetes mellitus treated with insulin • Ongoing or previous medically treated depression or bipolar disorder • Known autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis or multiple sclerosis) • Incompensated liver cirrhosis • Severe kidney disease (receiving dialysis) • Previous or other current cancer • Known medically treated sleep disorder (insomnia, restless legs etc.) • Shift-work or night-work • Daily intake of more than 5 units (1 unit = 8 g pure alcohol) • Pre-operative, continuous treatment with psychopharmacological drugs of any kind, opiods, anxiolytics or hypnotics • Predicted bad compliance • Breast-feeding • Pre-operative Mini Mental State Evaluation (MMSE) score less than 24

Approximately 1 week preoperatively patients are individually assessed and screened for inclusion. This includes a MMSE, a neuropsychological test with the ISPOCD test battery [13] and the MDI [14-16]. Hereafter, patients will on a daily basis fill out visual analogue scales (VAS) regarding anxiety, fatigue, pain, general well-being and sleep, complete a sleep diary and fill out the Karolinska Sleepiness Scale (KSS). An Actigraph will be mounted on the wrist and worn continuously until two weeks postoperatively. Patients will be randomized to either 6 mg (2x3mg) melatonin or placebo. Patients will be instructed to take their medicine every evening at 9-10 p.m. and continue with this for 13 weeks. A blood sample will be collected for the analysis of PER3 genotype.

A visit is scheduled 2 weeks postoperatively where patients are tested with the ISPOCD test battery and the MDI. At this time the Actigraph will be taken off.

During the last 10 weeks of the study patients will be assessed with the MDI twice, and every two weeks fill out visual analogue scales (VAS) regarding anxiety, fatigue, pain, general well-being and sleep, complete a sleep diary and fill out the Karolinska Sleepiness Scale (KSS).

At the final visit 12 weeks postoperatively patients will be tested with the ISPOCD test battery and the MDI.

Randomization

Patients who have provided written and informed consent preceding inclusion and who meet all the inclusion criteria and none of the exclusion criteria are randomized to either melatonin 6 mg or placebo. Inclusion and randomization is done 1 week preoperatively.

The randomization is based on blocks of 6. The randomization list is computer generated using dedicated software (www.randomization.com). To ensure that the randomization list is not known to the investigator this procedure is completed by the pharmacy who receives the medicine directly from Pharma Nord ApS, Vejle, Denmark. In case of drop-outs leading to less than 120 patients in

each group, the study will include new blocks of 6 patients to ensure randomization and balance between the two groups.

Melatonin

Melatonin is a hormone which is produced at night in the corpus pineale in a rhythmical pattern, controlled by an endogenous clock in the suprachiasmatic nucleus of the hypothalamus [17]. Its main function is to synchronize the circadian rhythm [17]. Melatonin is mostly known for its role as a circadian hormone but it also has known sedative [18;19], anxiolytic [18;19], analgesic [20;21], antihypertensive [22;23], non-inflammatory [24] and oncostatic effects [25-27]. Melatonin has a possible antidepressive effect [28-34] probably based on its effect on the central circadian regulation [35] and an effect on improving cognitive function [36]. Figure 1 shows the complexity of the relationship between breast cancer and depression and the possible attack points of melatonin.

The secretion rhythm, fluctuations and levels of melatonin have been previously investigated with regards to surgery, depression and also breast cancer. After surgery melatonin secretion has shown to be acutely disturbed [37] with a delay of secretion and reduced amplitude[38;39]. Various lines of evidence show that depressed patients exhibit disturbances in both the amplitude and the shape of the melatonin secretion rhythm, with some studies showing a low nocturnal melatonin secretion and others showing an increase in secretion [35]. A growing amount of evidence from prospective studies suggest an association between night work and breast cancer risk, most likely due to subsequent melatonin suppression [40].

Melatonin is relatively non-toxic [41-43]. Animal studies have shown that the maximum dose given in vivo, without any adverse effects or death is 200 mg/kg for pregnant rats throughout the whole pregnancy and 800 mg/kg for mice [44;45]. The drug has been used in many clinical studies of both adults [18-20;22;41;42;46;47] and newborns [48;49] without serious adverse effects. Doses of 1000 mg daily for one month have been given and the only reported adverse effect was drowsiness [41]. In a recent systematic review the most often reported side effects were headache, dizziness, nausea and drowsiness [50].

PER3 clock-gene

Previous studies have shown that certain genes, called clock-genes, have a role in regulating circadian rhythms and sleep in humans [51]. A coding region in the clock-gene PER3, which is repeated in either 4 or 5 units has been coupled to various phenotypical traits; A or B people, sleep diseases [52-54], affective disorders [55;56], cognitive function after sleep deprivation [54;57] and in one

study [58] a relationship was found between the 5/5 og 4/5 genotype of the PER3 gene and breast cancer. We would like to investigate whether sleep quality, cognitive function or depressive symptoms are correlated with any of these three PER3 genotypes.

Ethics

The study will be performed in agreement with the Helsinki II declaration and law 503 of 1992 about the Scientific Ethics Committee System and is approved by the local ethics committee (H-4-2011-007). The study has also been approved by The Danish Medicines Agency (EudraCT nr. 2010-022460-12) and The Danish Data Protection Agency (2007-58-0015/HEH.750.89-12). The project is registered on www.clinicaltrials.gov as recommended by the International Committee of Medical Journal Editors - clinicaltrials.gov identifier: NCT01355523.

EFFECT PARAMETERS

Table 2: Effect parameters

PRIMARY EFFECT PARAMETER	SECONDARY EFFECT PARAMETERS	TERTIARY EFFECT PARAMETERS
• MDI (Major Depression Inventory)	• Anxiety measured by VAS • Minutes of sleep, sleep effeciency, sleep latency and periods awake measured by actigraphy • Changes in cognitive function measured by a neuropsychological test-battery • PER3 genotype correlated with sleep, cognitive function and depressive symptoms	• Fatigue, pain, well-being and sleep quality/quantity measured by VAS • Sleep diary • Sleepiness measured by the Karolinska Sleepinesss Scale

Major Depression Inventory (MDI)

Major Depression Inventory is a self-rating scale including 12 questions. The questionnaire is already well documented in a Danish population [14]. The questions cover the 10 ICD-10 questions for depression and the symptoms are identical with the DSM-IV major depression diagnosis apart from one symptom, low self esteem (question 4), which in DSM-IV is incorporated in the question about guilt (question 5). The MDI includes 10 items, where item 8 and 10 are divided into two sub-questions a and b. For item 8 and 10 the highest score of question a and b is included. The individual items are measured on a 6-point Likert scale with graduations depending on the extent of the symptom the last 14 days; 0 (the symptom has not been present) till 5 (the symptom has been present continuously).

The MDI has a dual function. It can be used as a diagnostic tool where it can, by algorithms, lead to either DSM-IV or ICD-10 categories of "major" or "moderate to severe depression". An acceptable sensitivity and specificity for the diagnosis of depression according to ICD-10 and DSM-III/DSM-IV has previously been found [15]. The MDI can also be used as a measuring instrument to indicate the severity of the depression. Thus, the MDI correlates with the Hamiltons Depressions Scale (HAM-D) [16].

Actigraphy

Actigraphy is a well known non-invasive method to objectively measure sleep. Using an Actigraph, activity levels are registered by a wrist-worn mini-computer. This method which does not disturb the patients' sleep has been used for many years [59] and also in the postoperative setting [60]. It has been shown that actigraphy has a high sensitivity and specificity for detecting sleep start, sleep periods and awakenings [59].

An actigraph measures small accelerations with a piezo-electrode and stores this in a memory. Data can be recorded via different modalities and we will be using the zero crossing method, where all accelerations (> 0.1G) which cross a certain threshold (0 or very close to 0) will be detected. The sleep analysis will be performed by dedicated software (Action4 software, Ambulatory Monitoring Inc., NY, USA) by using the Cole-Kripke algorithm [61]. Data will be reported as total sleep time per period, sleep efficiency, sleep ratio, number of awakenings and duration of awakenings. The method is validated and has been used previously to measure sleep in patients with breast cancer before, during and after surgery [62-65].

The ISPOCD neuropsychological test battery

The cognitive function of the patients will be tested preoperatively and at 2 weeks and 3 months postoperatively. The test battery consists of 4 validated tests: the Visual Verbal Learning Test, the Stroop Colour-Word Test, the Letter-Digit Coding Test and the Concept Shifting Task.

The test battery takes approximately 45 minutes to complete and has been used previously in the large multicenter ISPOCD trial [13].

Anxiety and sleep quality measurements

A subjective feeling of anxiety will be registered on a VAS going from "no anxiety" to "worst possible anxiety". Measurements will take place daily for the first three weeks of the study and every two weeks for the last 10 weeks.

Subjective sleep quality will be registered on a VAS going from "best possible sleep" equivalent to 0 mm to "worst possible sleep" equivalent to 100 mm. Patients will also fill out VAS regarding general well-being, fatigue and pain. Measurements will take place at the same time as for anxiety. Furthermore, a sleep diary recording sleep time and awakening time, will be completed daily for the first 3 weeks of the study and thereafter every 14 days. Patients will at the same time periods complete the KSS which is used to quantify levels of sleepiness. KSS is a 9 point scale from 1 (very awake) to 9 (very sleepy), where a score of 7 or more reflects pathological sleepiness [66].

Other collected data

Age, weight, height, menopausal status, educational level, cohabitation status, work market affiliation, household income, number of children/number of children living at home, smoking habits, ASA class, duration of surgery, the size of the incision, consumption of analgesics postoperatively and which postoperative oncological treatment each patient receives after surgery.

STATISTICAL ANALYSIS AND SAMPLE SIZE CALCULATION

The sample size estimation is based on a conservative estimate of the incidence of depression of 30% in breast cancer patients [7] with a reduction to 15% with melatonin treatment. With a power of 80%, a risk of type I error of 5% and a risk of type II error of 20% we should include 120 patients in each group receiving melatonin or placebo. We have chosen to include 130 patients in each group. Statistical analyses will be done using SPSS Version 18.0.

Regarding our primary outcome mean MDI total scores and prevalences of ICD-10 mild, moderate and severe depression will be calculated for both samples. Normality of the data will be tested by one sample Kolmogorov-Smirnov test and parametric or non-parametric statistics will be used accordingly. The two groups will be compared by Fisher's exact test for the primary outcome. Paired Student's t-test or Wilcoxon test will be used for intra-group comparisons and unpaired Student's t-test or Mann-Whitney's test for inter-group comparisons. For repeated measures Friedman test or an ANOVA will be used when appropriate.

For comparing anxiety by VAS and sleep architecture (objective data from the Actigraph) we will do inter-group comparisons with Mann-Whitney's test and intra-group comparisons using the Wilcoxon signed rank test.

For analyzing POCD in the two groups we will use 2x2 tables and a Fisher's exact test. Patients will be defined as having POCD if 2 of the seven Z-scores in individual test or the combined Z-score are 1.96 or more [13]. ANOVA will be used to analyze the 2 groups and the specific Z-scores for the

seven subtests and the combined Z-scores. A Bonferroni correction will be made when performing multiple comparisons.

For analyses of correlation between PER3 genotype and sleep, cognitive function and depressive symptoms a logistic regression analysis will be used. For the subjective parameters fatigue, pain, general wellbeing and sleep quality/quantity measured by VAS and sleepiness by KSS we will do inter-group comparisons with the Mann Whitney test and intra-group comparisons using the Wilcoxon signed rank test. We plan to do intention-to-treat as well as per protocol analyses. In general, $p<0.05$ will be considered statistically significant.

DISCUSSION

There is a complex relationship between depression and breast cancer with influence from various factors – figure 1. Due to the high frequencies of depression, anxiety, sleep disturbances and cognitive dysfunction in patients with breast cancer, a high interest is found in preventing these co-morbid symptoms.

Depression is both underdiagnosed and undertreated in many cancer patients [11;67], especially those with breast cancer [68]. The overall rate of depression in patients with breast cancer is higher than in most cancers. This is most likely because menopause, either naturally occurring or premature due to the effects of chemotherapy and/or anti-hormone treatment, and estrogen decline are related to depression [6]. It has been shown that up to 50 % of patients with breast cancer may experience depression and/or anxiety within the first year of diagnosis [7]. Concomitant breast cancer and depression is associated with higher mortality and morbidity [67;69-72] but also lower patient satisfaction [73] and compliance to adjuvant therapy [74] and general medical treatment [75]. Even a year after surgery many women still deal with an anxiety problem [76] and about 15 % are still depressed [7]. Studies have shown that treating anxiety and depression in these patients with breast cancer improves their quality of life, leads to a higher completion of adjuvant therapy and extends their lifetime [68;77;78]. Therefore it is important to optimize the treatment of these symptoms.

A number of randomized controlled trials have examined the efficacy of antidepressants compared with placebo in patients with breast cancer [78-85]. A high number of drop-outs due to side effects related to the antidepressant treatment have also rendered such trials difficult to complete [79;84;86]. Furthermore, recent evidence indicates that some selective serotonin reuptake inhibitor antidepressants may reduce tamoxifen's effectiveness and are associated with an increased risk of mortality [87;88].

Sleep disturbances are a frequent problem in cancer patients [89;90]. Compared with other types of cancer breast cancer is associated with an exceptionally high rate of reduced sleep quality [10;89] which can be found even many years after end of treatment [8]. The estimated prevalence of sleep problems among patients with breast cancer is between 38-61% [10;89;91;92], and it may reduce quality of life in this group of patients [91;93]. About 30 % of patients with breast cancer take hypnotics [10;89;92] leading to a potential dependency problem and it is therefore important to focus on treatments with less adverse effects.

The sleep disturbances can be due to a variety of factors, both physical and psychological, which all have a mutual influence on each other. Among the psychological factors, depression and anxiety are well-known and these patients also exhibit a change in sleep architecture [94]. In general, depressed patients display sleep abnormalities such as difficulties falling asleep and staying asleep, lose of slow-wave-sleep and changed REM sleep [95]. Pain and hot flushes are examples of the physical factors [8;91;92].

Overall, it is difficult to conclude whether sleep disturbances are a precursor or a sequelae of depression. Various studies in healthy individuals have shown a causal relationship between sleep disturbances and the following development of depression [96;97]. More specifically for patients with breast cancer, a circadian rhythm disruption has been associated with depression, fatigue and pain [62;63;65;98]. Altogether there is a complicated relationship between circadian disturbances, sleep and mood [35] and this is especially prevalent in patients with breast cancer [99].

Disturbances of cognitive function are a prevalent phenomenon in patients with breast cancer and can influence the general quality of life in this group of patients [9;12]. Studies have suggested that the cancer *per se* and/or the treatment with surgery, radiation, chemotherapy and hormone therapy or genetics can be contributing factors in the development of cognitive disturbances [100;101]. POCD (postoperative cognitive dysfunction) is characterized by a deterioration in memory, concentration and information assessment after surgery [13].

In other settings some studies have shown that melatonin can attenuate cognitive dysfunction [36;102;103] and this mechanism together with the general improvement of sleep could be beneficial on cognitive disturbances in this specific group of patients. Since there is no specific knowledge on this topic with regards to breast cancer it is necessary to investigate whether the development of cognitive problems can be prevented by melatonin treatment.

In conclusion we hope, with this project, to decrease the occurrence of depression, anxiety, sleep disturbances and cognitive dysfunction in patients with breast cancer and in a larger perspective reduce morbidity and mortality and improve quality of life for these patients. With regards to genetics this project could lead to the possibility of being able to detect women with a higher risk of developing the abovementioned problems and then give indication for selective prophylactic treatment. The diversity of melatonin's physiological functions and treatment effects are continuously being investigated in both animal and human studies. To-date, the effect of melatonin in a breast cancer population with the above-mentioned indications has not yet been studied.

Conflict of interest statement:
No conflicts of interest.

Funding statement:
This work was supported by grants from the University of Copenhagen, The Aase and Ejnar Danielsens Foundation, The A.P.Møller Foundation for the Advancement of Medical Science, The Else and Mogens Wedell Wedellborgs Foundation, The Beckett Foundation, The Hede Nielsen Family Foundation, The Dagmar Marshalls Foundation and Manufacturer Einar Willumsen's Memorial Scholarship. Pharma Nord provided the melatonin and placebo tablets but had no influence on the study design and will have no influence on the interpretation of results.

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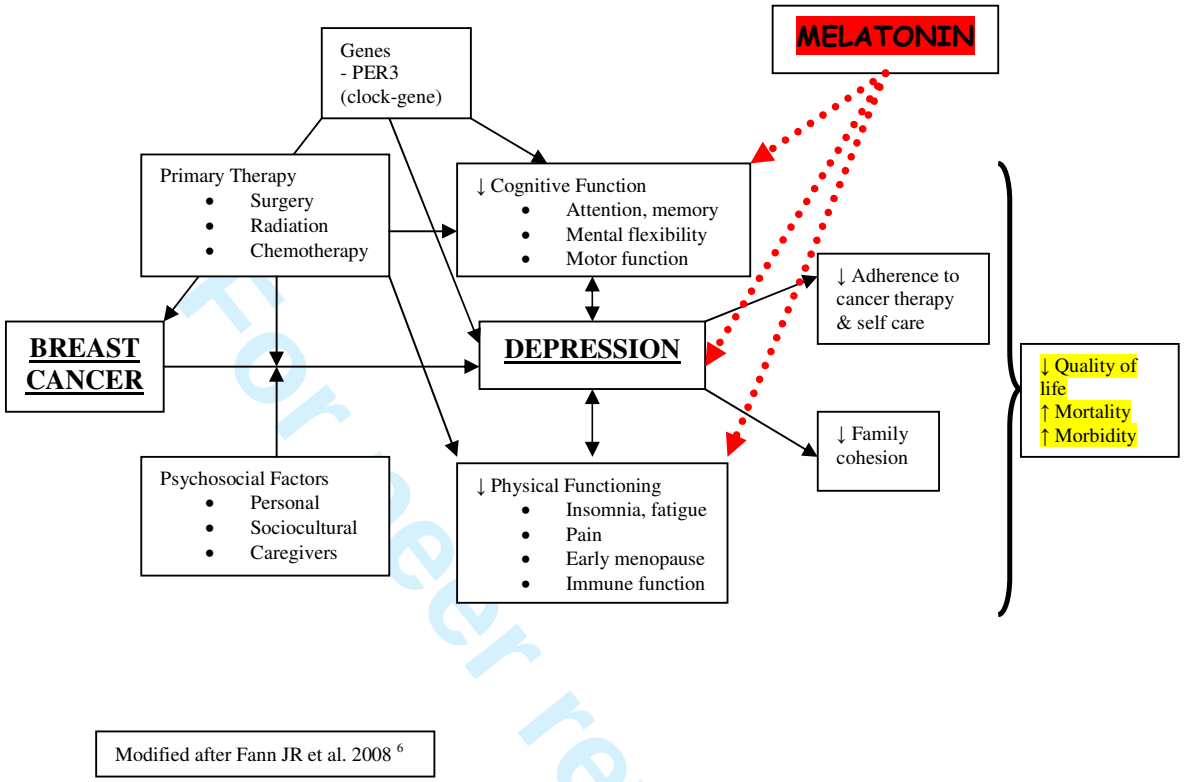
Figure 1: The relationship between breast cancer and depression



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-5
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3-4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	3-4
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	3-4
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4,6-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	8-8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			4-5
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	4-5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	4-5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4-5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	3

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	4-5
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	4-5
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	1-2
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	11

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

The effect of MELatOnin on Depression, anxietY, cognitive function and sleep disturbances in patients with breast cancer. The MELODY trial: protocol for a randomized, placebo-controlled, double-blinded trial.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000647.R1
Article Type:	Protocol
Date Submitted by the Author:	15-Dec-2011
Complete List of Authors:	Hansen, Melissa; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section Madsen, Michael; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section Hageman, Ida; Rigshospitalet, University of Copenhagen, Psychiatric Center Copenhagen Rasmussen, Lars; Rigshospitalet, University of Copenhagen, Department of Anaesthesia, Centre of Head and Orthopaedics Bokmand, Susanne; Herlev Hospital, University of Copenhagen, Department of Breast Surgery Rosenberg, Jacob; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section Gögenur, Ismail; Herlev Hospital, University of Copenhagen, Department of Gastroenterology, Surgical Section
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Oncology, Pharmacology & therapeutics, Mental health
Keywords:	Breast surgery < SURGERY, Depression & mood disorders < PSYCHIATRY, Delirium & cognitive disorders < PSYCHIATRY, SLEEP MEDICINE, Anxiety disorders < PSYCHIATRY, Clinical trials < THERAPEUTICS

Title page – study protocol

The effect of MELatOnin on Depression, anxietY, cognitive function and sleep disturbances in patients with breast cancer. The MELODY trial: protocol for a randomized, placebo-controlled, double-blinded trial.

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Key words: breast cancer, melatonin, depression, sleep disturbances, cognitive function

Word count: 4012

ABSTRACT

Introduction: Breast cancer represents about one third of all cancer diagnoses and accounts for about 15 % of cancer deaths in women. Many of these patients experience depression, anxiety, sleep disturbances and cognitive dysfunction. This may adversely affect quality of life but also contribute to morbidity and mortality. Melatonin is a regulatory circadian hormone having, among others, a hypnotic and an anti-depressive effect. It has very low toxicity and very few adverse effects compared with the more commonly used antidepressants and hypnotics.

Methods and analysis: The objective of this double-blind randomized, placebo-controlled trial is to investigate whether treatment with oral melatonin has a prophylactic or ameliorating effect on depressive symptoms, anxiety, sleep disturbances and cognitive dysfunction in women with breast cancer. Furthermore we will examine whether a specific clock-gene PER3 is correlated with an increased risk of depressive symptoms, sleep disturbances or cognitive dysfunction.

The MELODY-trial is a prospective double-blinded, randomized, placebo-controlled trial in which we intend to include 260 patients. The primary outcome is depressive symptoms measured by the Major Depression Inventory (MDI). The secondary outcomes are anxiety measured by a visual analogue scale (VAS), total sleep time, sleep efficiency, sleep latency and periods awake measured by actigraphy and changes in cognitive function measured by a neuropsychological test-battery. Tertiary outcomes are fatigue, pain, well-being and sleep quality/quantity measured by VAS, sleep diary and sleepiness measured by the Karolinska Sleepiness Scale. The PER3 genotype is also to be determined in blood samples.

Ethics: The study is approved by the local ethics committee (H-4-2011-007). The study has also been approved by The Danish Medicines Agency (EudraCT nr. 2010-022460-12) and The Danish Data Protection Agency (2007-58-0015/HEH.750.89-12). The project is registered on www.clinicaltrials.gov - clinicaltrials.gov identifier: NCT01355523.

INTRODUCTION

Breast cancer is the most common type of cancer among women worldwide with about 1.4 million new cases every year [1]. Breast cancer accounts for 31 % of the various cancer diagnoses in women and is responsible for 15 % of deaths in females due to cancer [2]. For Danish women it is the most common type of cancer and the incidence is rising [3]. The incidence rises towards the age of 65 and then the curve flattens out [3]. Improvement of treatment has led to increasing 5 year-survival which is now 84 % in Denmark [4] and 89 % in the US [5]. Now focus has turned to optimizing quality of life because these patients may have a number of different psychological and physical symptoms such as depression, anxiety, fatigue, cognitive dysfunction and sleep disturbances [6-12].

METHODS

Study design and objectives

MELODY (The effect of **MEL**at**O**nin on **D**epression, anxiet**Y**, cognitive function and sleep disturbances in breast cancer patients) is a prospective double-blinded, randomized, placebo-controlled trial in which we intend to include 260 patients undergoing surgery for breast cancer at Herlev University Hospital in Copenhagen, Denmark. If target sample size is not being adequately achieved, other centres/hospitals in Denmark will be invited to participate.

The objective is to investigate whether treatment with oral melatonin has a prophylactic or ameliorating effect on depressive symptoms, anxiety, sleep disturbances and cognitive dysfunction in women with breast cancer. Furthermore, we will examine whether a specific clock-gene PER3 is correlated with an increased risk of depressive symptoms, sleep disturbances or cognitive function.

Patients in the intervention group will receive 6 mg oral melatonin daily 1 hour before bedtime for 1 week preoperatively to 12 weeks postoperatively. Patients in the control group will receive placebo. The melatonin/placebo will be supplied from Pharma Nord ApS, Vejle, Denmark and the tablets (melatonin/placebo) are physically identical.

Study population

Table 1: Inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
• Female between 30-75 years • Lumpectomy or mastectomy • American Society of Anesthesiologists (ASA) class I-III • No sign of depression on Major Depression Inventory (MDI) • Not pregnant	• Neoadjuvant chemotherapy • Treatment with: - Selective serotonin reuptake inhibitors (SSRI) - Antithrombotic drug therapy (except 75 mg acetylsalicylic acid (ASA) daily) - Monoaminoxidase (MAO) inhibitors - Calcium channel blockers • Rotor or Dubin-Johnson syndrome • Epilepsy • Known allergic reaction to melatonin • Known and treated sleep apnea • Diabetes mellitus treated with insulin • Ongoing or previous medically treated depression or bipolar disorder • Known autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis or multiple sclerosis) • Incompensated liver cirrhosis • Severe kidney disease (receiving dialysis) • Previous or other current cancer • Known medically treated sleep disorder (insomnia, restless legs etc.) • Shift-work or night-work • Daily intake of more than 5 units (1 unit = 8 g pure alcohol) • Pre-operative, continuous treatment with psychopharmacological drugs of any kind, opiods, anxiolytics or hypnotics • Predicted poor compliance • Breast-feeding • Pre-operative Mini Mental State Evaluation (MMSE) score less than 24

Approximately 1 week preoperatively patients are individually assessed and screened for inclusion. This includes a MMSE, a neuropsychological test with the ISPOCD test battery [13] and the MDI [14-16]. Hereafter, patients will on a daily basis fill out visual analogue scales (VAS) regarding anxiety, fatigue, pain, general well-being and sleep, complete a sleep diary and fill out the Karolinska Sleepiness Scale (KSS). An Actigraph will be mounted on the wrist and worn continuously until two weeks postoperatively. Patients will be randomized to either 6 mg (2x3mg) melatonin or placebo. Patients will be instructed to take their medicine every evening at 9-10 p.m. and continue with this for 13 weeks. A blood sample will be collected for the analysis of PER3 genotype.

A visit is scheduled 2 weeks postoperatively where patients are tested with the ISPOCD test battery and the MDI. At this time the Actigraph will be taken off.

During the last 10 weeks of the study patients will be assessed with the MDI twice, and every two weeks fill out visual analogue scales (VAS) regarding anxiety, fatigue, pain, general well-being and sleep, complete a sleep diary and fill out the Karolinska Sleepiness Scale (KSS).

At the final visit 12 weeks postoperatively patients will be tested with the ISPOCD test battery and the MDI.

To ensure compliance and promote participant retention and follow-up patients will throughout the whole study period of 13 weeks be contacted by telephone 7 times to remind them to fill out VAS, sleep diary, KSS and MDI at appropriate times, to remind them to take their tablets daily, to ask about adverse reactions and to ask about diagnosis of depression since the last contact.

Criteria for discontinuing in the trial

During the trial patients who experience serious peri- or postoperative complications/events, causing unexpected morbidity or pain during the first postoperative days (i.e. cardiopulmonary complications (myocardial infarction, serious arrhythmia, pulmonary oedema), thromboembolic complications (deep venous thrombosis, pulmonary embolism), wound infection (with fever and elevated WBC's and CRP) will be excluded. No further data will be collected on these patients after they are excluded, previously collected data will not be analysed and the patients will not continue the trial medication.

The goal with medicine compliance is a minimum of 75 % compliance in the first 3 weeks and 50 % for the rest of the study period. Patients will be excluded if they at the visit 2 weeks postoperatively have not taken at least 75 % of the study medication. No further data will be collected and the patients will not continue the trial medication. Patients who have not taken at least 50 % of the trial medication at the last visit will not be analysed on the long-term effect variables. Patients will be instructed to bring all the study medication (open and closed blister packets) with them at every visit to control compliance.

All potential, included and randomised patients will be accounted for in a screenings log, in an identification list, in the CONSORT trial profile and in a baseline data table.

Randomization

Patients who have provided written and informed consent preceding inclusion and who meet all the inclusion criteria and none of the exclusion criteria are randomized to either melatonin 6 mg or placebo. Inclusion and randomization is done 1 week preoperatively.

The randomization is based on blocks of 6. The randomization list is computer generated using dedicated software (www.randomization.com). To ensure that the randomization list is not known to the investigator this procedure is completed by the pharmacy who receives the medicine directly from Pharma Nord ApS, Vejle, Denmark. In case of drop-outs leading to less than 120 patients in

each group, the study will include new blocks of 6 patients to ensure randomization and balance between the two groups.

Data and adverse events

Data is collected on individual paper case report forms (CRF) and data will be stored for 15 years and then destroyed. Data from these CRF's will be transferred as double data entry to a database. In this database patients are coded with a patient number and the database is saved on the hospitals computer server to ensure maximum security. Spot checks to validate data transfer from the CRF's to the electronic database will be completed by The Good Clinical Practice Unit.

Due to melatonins relative non-toxicity shown in both animal and human studies and due to the fact that prolonged treatment with melatonin or melatonin receptor agonists have not shown any serious adverse effects, no Data Monitoring Committee is initiated and no interim analyses have been planned in the course of the study.

Throughout the trial both the sponsor/investigator, who informs the patient and the patient herself, will be blinded. A code breach will occur if an unexpected event arises in relation to the surgical procedure and/or the postoperative period where there is a reasonable probability of an adverse reaction. Unexpected events do not include procedure-related surgical/medical/anaesthetic complications. Code breach is possible without prior contact to sponsor/investigator. The risk of early trial termination is not existent.

The summary of product characteristics (SPC) for melatonin will be used as a reference document. The following known, usual side effects (1-10%) and non usual side effects (0.1-1%) will not be registered as adverse events: light headache, light nausea, dyspepsia, minor symptoms of sleepiness and light morning-drowsiness. Furthermore complications in relation to surgery will neither be registered as adverse events.

All adverse events will be registered and reported to The Danish Medicines Agency and the local ethics committee in the final report. All serious adverse events will be reported to the local ethics committee in an annual report together with a report on patient safety. All serious adverse reactions will be reported to The Danish Medicines Agency in an annual report together with a report on patient safety. The SPC for melatonin will be used to judge whether a serious adverse reaction is expected/unexpected and thereby a possible sudden unexpected serious adverse reaction. The sponsor/investigator will make sure that all information about sudden unexpected serious adverse reaction, that are lethal or life-threatening will be registered and reported to The Danish Medicines Agency as soon as possible and at the latest 7 days after sponsor/investigator has received

knowledge of such a reaction. At the latest 8 days after this reporting, sponsor/investigator will inform The Danish Medicines Agency of the follow-up. All other sudden unexpected serious adverse reactions will be reported to The Danish Medicines Agency at the latest 15 days after sponsor/investigator has gained knowledge of these. In these situations the patient will be followed until the reaction has terminated – either via contact with the sponsor/investigator or via the outpatient clinic at the hospital.

Melatonin

Melatonin is a hormone which is produced at night in the corpus pineale in a rhythmical pattern, controlled by an endogenous clock in the suprachiasmatic nucleus of the hypothalamus [17]. Its main function is to synchronize the circadian rhythm [17]. Melatonin is mostly known for its role as a circadian hormone but it also has known sedative [18;19], anxiolytic [18;19], analgesic [20;21], antihypertensive [22;23], non-inflammatory [24] and oncostatic effects [25-27]. Melatonin has a possible antidepressive effect [28-34] probably based on its effect on the central circadian regulation [35] and an effect on improving cognitive function [36]. Figure 1 shows the complexity of the relationship between breast cancer and depression and the possible attack points of melatonin.

The secretion rhythm, fluctuations and levels of melatonin have been previously investigated with regards to surgery, depression and also breast cancer. After surgery melatonin secretion has shown to be acutely disturbed [37] with a delay of secretion and reduced amplitude[38;39]. Various lines of evidence show that depressed patients exhibit disturbances in both the amplitude and the shape of the melatonin secretion rhythm, with some studies showing a low nocturnal melatonin secretion and others showing an increase in secretion [35]. A growing amount of evidence from prospective studies suggest an association between night work and breast cancer risk, most likely due to subsequent melatonin suppression [40].

Melatonin is relatively non-toxic [41-43]. Animal studies have shown that the maximum dose given in vivo, without any adverse effects or death is 200 mg/kg for pregnant rats throughout the whole pregnancy and 800 mg/kg for mice [44;45]. The drug has been used in many clinical studies of both adults [18-20;22;41;42;46;47] and newborns [48;49] without serious adverse effects. Doses of 1000 mg daily for one month have been given and the only reported adverse effect was drowsiness [41]. In a recent systematic review the most often reported side effects were headache, dizziness, nausea and drowsiness [50].

PER3 clock-gene

Previous studies have shown that certain genes, called clock-genes, have a role in regulating circadian rhythms and sleep in humans [51]. A coding region in the clock-gene PER3, which is repeated in either 4 or 5 units has been coupled to various phenotypical traits; A or B people, sleep diseases [52-54], affective disorders [55;56], cognitive function after sleep deprivation [54;57] and in one study [58] a relationship was found between the 5/5 og 4/5 genotype of the PER3 gene and breast cancer. We would like to investigate whether sleep quality, cognitive function or depressive symptoms are correlated with any of these three PER3 genotypes.

Ethics

The study will be performed in agreement with the Helsinki II declaration and law 503 of 1992 about the Scientific Ethics Committee System and is approved by the local ethics committee (H-4-2011-007). The study has also been approved by The Danish Medicines Agency (EudraCT nr. 2010-022460-12) and The Danish Data Protection Agency (2007-58-0015/HEH.750.89-12). The project is registered on www.clinicaltrials.gov as recommended by the International Committee of Medical Journal Editors - clinicaltrials.gov identifier: NCT01355523. The Good Clinical Practice Unit at Copenhagen University will oversee the trial and conduct trial audit periodically.

All authors will have direct access to data during and after the trial. Furthermore the sponsor investigator will allow direct access to source data/documents, including patient charts, at monitoring, audit and/or inspection from The Danish Medicines Agency, The Good Clinical Practice Unit or from health authorities from other countries.

EFFECT PARAMETERS

Table 2: Effect parameters

PRIMARY EFFECT PARAMETER	SECONDARY EFFECT PARAMETERS	TERTIARY EFFECT PARAMETERS
• MDI (Major Depression Inventory)	• Anxiety measured by VAS • Total sleep time, sleep efficiency, sleep latency and periods awake measured by actigraphy • Changes in cognitive function measured by a neuropsychological test-battery • PER3 genotype correlated with sleep, cognitive function and depressive symptoms	• Fatigue, pain, well-being and sleep quality/quantity measured by VAS • Sleep diary • Sleepiness measured by the Karolinska Sleepinesss Scale

Major Depression Inventory (MDI)

Major Depression Inventory is a self-rating scale including 12 questions. The questionnaire is already well documented in a Danish population [14]. The questions cover the 10 ICD-10 questions for depression and the symptoms are identical with the DSM-IV major depression diagnosis apart from one symptom, low self esteem (question 4), which in DSM-IV is incorporated in the question about guilt (question 5). The MDI includes 10 items, where item 8 and 10 are divided into two sub-questions a and b. For item 8 and 10 the highest score of question a and b is included. The individual items are measured on a 6-point Likert scale with graduations depending on the extent of the symptom the last 14 days; 0 (the symptom has not been present) till 5 (the symptom has been present continuously).

The MDI has a dual function. It can be used as a diagnostic tool where it can, by algorithms, lead to either DSM-IV or ICD-10 categories of "major" or "moderate to severe depression". An acceptable sensitivity and specificity for the diagnosis of depression according to ICD-10 and DSM-III/DSM-IV has previously been found [15]. The MDI can also be used as a measuring instrument to indicate the severity of the depression. Thus, the MDI correlates with the Hamiltons Depressions Scale (HAM-D) [16].

Actigraphy

Actigraphy is a well known non-invasive method to objectively measure sleep. Using an Actigraph, activity levels are registered by a wrist-worn mini-computer. This method which does not disturb the patients' sleep has been used for many years [59] and also in the postoperative setting [60]. It has been shown that actigraphy has a high sensitivity and specificity for detecting sleep start, sleep periods and awakenings [59]. The gold standard for measuring sleep is polysomnography. Actigraphy has a high specificity for detecting whether the patient is asleep or awake but can not differentiate between sleep stages and score REM sleep.

An actigraph measures small accelerations with a piezo-electrode and stores this in a memory. Data can be recorded via different modalities and we will be using the zero crossing method, where all accelerations (> 0.1G) which cross a certain threshold (0 or very close to 0) will be detected. The sleep analysis will be performed by dedicated software (Action4 software, Ambulatory Monitoring Inc., NY, USA) by using the Cole-Kripke algorithm [61]. Data will be reported as total sleep time per period, sleep efficiency, sleep ratio, number of awakenings and duration of awakenings. The method is validated and has been used previously to measure sleep in patients with breast cancer before, during and after surgery [62-65].

The ISPOCD neuropsychological test battery

The cognitive function of the patients will be tested preoperatively and at 2 weeks and 3 months postoperatively. The test battery consists of 4 validated tests: the Visual Verbal Learning Test, the Stroop Colour-Word Test, the Letter-Digit Coding Test and the Concept Shifting Task.

The test battery takes approximately 45 minutes to complete and has been used previously in the large multicenter ISPOCD trial [13].

Anxiety and sleep quality measurements

A subjective feeling of anxiety will be registered on a VAS going from "no anxiety" to "worst possible anxiety". Measurements will take place daily for the first three weeks of the study and every two weeks for the last 10 weeks.

Subjective sleep quality will be registered on a VAS going from "best possible sleep" equivalent to 0 mm to "worst possible sleep" equivalent to 100 mm. Patients will also fill out VAS regarding general well-being, fatigue and pain. Measurements will take place at the same time as for anxiety. Furthermore, a sleep diary recording sleep time and awakening time, will be completed daily for the first 3 weeks of the study and thereafter every 14 days. Patients will at the same time periods complete the KSS which is used to quantify levels of sleepiness. KSS is a 9 point scale from 1 (very awake) to 9 (very sleepy), where a score of 7 or more reflects pathological sleepiness [66].

Other collected data

Age, weight, height, menopausal status, educational level, cohabitation status, work market affiliation, household income, number of children/number of children living at home, smoking habits, ASA class, duration of surgery, the size of the incision, consumption of analgesics postoperatively and which postoperative oncological treatment each patient receives after surgery.

STATISTICAL ANALYSIS AND SAMPLE SIZE CALCULATION

The sample size estimation is based on a conservative estimate of the incidence of depression of 30% in breast cancer patients [7] with a reduction to 15% with melatonin treatment. With a power of 80%, a risk of type I error of 5% and a risk of type II error of 20% we should include 120 patients in each group receiving melatonin or placebo. We have chosen to include 130 patients in each group.

Statistical analyses will be done using SPSS Version 18.0.

Regarding our primary outcome mean MDI total scores and prevalences of ICD-10 mild, moderate and severe depression will be calculated for both samples. Normality of the data will be tested by

one sample Kolmogorov-Smirnov test and parametric or non-parametric statistics will be used accordingly. The two groups will be compared by Fisher's exact test for the primary outcome. Paired Student's t-test or Wilcoxon test will be used for intra-group comparisons and unpaired Student's t-test or Mann-Whitney's test for inter-group comparisons. For repeated measures Friedman test or an ANOVA will be used when appropriate.

For comparing anxiety by VAS and sleep architecture (objective data from the Actigraph) we will do inter-group comparisons with Mann-Whitney's test and intra-group comparisons using the Wilcoxon signed rank test.

For analyzing POCD in the two groups we will use 2x2 tables and a Fisher's exact test. Patients will be defined as having POCD if 2 of the seven Z-scores in individual test or the combined Z-score are 1.96 or more [13]. ANOVA will be used to analyze the 2 groups and the specific Z-scores for the seven subtests and the combined Z-scores. A Bonferroni correction will be made when performing multiple comparisons.

For analyses of correlation between PER3 genotype and sleep, cognitive function and depressive symptoms a logistic regression analysis will be used. For the subjective parameters fatigue, pain, general wellbeing and sleep quality/quantity measured by VAS and sleepiness by KSS we will do inter-group comparisons with the Mann Whitney test and intra-group comparisons using the Wilcoxon signed rank test. We plan to do intention-to-treat as well as per protocol analyses. In general, $p<0.05$ will be considered statistically significant.

DISCUSSION

There is a complex relationship between depression and breast cancer with influence from various factors – figure 1. Due to the high frequencies of depression, anxiety, sleep disturbances and cognitive dysfunction in patients with breast cancer, a high interest is found in preventing these co-morbid symptoms.

Depression is both underdiagnosed and undertreated in many cancer patients [11;67], especially those with breast cancer [68]. The overall rate of depression in patients with breast cancer is higher than in most cancers. This is most likely because menopause, either naturally occurring or premature due to the effects of chemotherapy and/or anti-hormone treatment, and estrogen decline are related to depression [6]. It has been shown that up to 50 % of patients with breast cancer may experience depression and/or anxiety within the first year of diagnosis [7]. Concomitant breast cancer and depression is associated with higher mortality and morbidity [67;69-72] but also lower patient

satisfaction [73] and compliance to adjuvant therapy [74] and general medical treatment [75]. Even a year after surgery many women still deal with an anxiety problem [76] and about 15 % are still depressed [7]. Studies have shown that treating anxiety and depression in these patients with breast cancer improves their quality of life, leads to a higher completion of adjuvant therapy and extends their lifetime [68;77;78]. Therefore it is important to optimize the treatment of these symptoms.

A number of randomized controlled trials have examined the efficacy of antidepressants compared with placebo in patients with breast cancer [78-85]. A high number of drop-outs due to side effects related to the antidepressant treatment have also rendered such trials difficult to complete [79;84;86]. Furthermore, recent evidence indicates that some selective serotonin reuptake inhibitor antidepressants may reduce tamoxifen's effectiveness and are associated with an increased risk of mortality [87;88].

Sleep disturbances are a frequent problem in cancer patients [89;90]. Compared with other types of cancer breast cancer is associated with an exceptionally high rate of reduced sleep quality [10;89] which can be found even many years after end of treatment [8]. The estimated prevalence of sleep problems among patients with breast cancer is between 38-61% [10;89;91;92], and it may reduce quality of life in this group of patients [91;93]. About 30 % of patients with breast cancer take hypnotics [10;89;92] leading to a potential dependency problem and it is therefore important to focus on treatments with less adverse effects.

The sleep disturbances can be due to a variety of factors, both physical and psychological, which all have a mutual influence on each other. Among the psychological factors, depression and anxiety are well-known and these patients also exhibit a change in sleep architecture [94]. In general, depressed patients display sleep abnormalities such as difficulties falling asleep and staying asleep, lose of slow-wave-sleep and changed REM sleep [95]. Pain and hot flushes are examples of the physical factors [8;91;92].

Overall, it is difficult to conclude whether sleep disturbances are a precursor or a sequelae of depression. Various studies in healthy individuals have shown a causal relationship between sleep disturbances and the following development of depression [96;97]. More specifically for patients with breast cancer, a circadian rhythm disruption has been associated with depression, fatigue and pain [62;63;65;98]. Altogether there is a complicated relationship between circadian disturbances, sleep and mood [35] and this is especially prevalent in patients with breast cancer [99].

Disturbances of cognitive function are a prevalent phenomenon in patients with breast cancer and can influence the general quality of life in this group of patients [9;12]. Studies have suggested that the

cancer *per se* and/or the treatment with surgery, radiation, chemotherapy and hormone therapy or genetics can be contributing factors in the development of cognitive disturbances [100;101]. POCD (postoperative cognitive dysfunction) is characterized by a deterioration in memory, concentration and information assessment after surgery [13].

In other settings some studies have shown that melatonin can attenuate cognitive dysfunction [36;102;103] and this mechanism together with the general improvement of sleep could be beneficial on cognitive disturbances in this specific group of patients. Since there is no specific knowledge on this topic with regards to breast cancer it is necessary to investigate whether the development of cognitive problems can be prevented by melatonin treatment.

In conclusion we hope, with this project, to decrease the occurrence of depression, anxiety, sleep disturbances and cognitive dysfunction in patients with breast cancer and in a larger perspective reduce morbidity and mortality and improve quality of life for these patients. With regards to genetics this project could lead to the possibility of being able to detect women with a higher risk of developing the abovementioned problems and then give indication for selective prophylactic treatment. The diversity of melatonin's physiological functions and treatment effects are continuously being investigated in both animal and human studies. To-date, the effect of melatonin in a breast cancer population with the above-mentioned indications has not yet been studied.

Conflict of interest statement:

No conflicts of interest.

Funding statement:

This work was supported by grants from the University of Copenhagen, The Aase and Ejnar Danielsens Foundation, The A.P.Møller Foundation for the Advancement of Medical Science, The Else and Mogens Wedell Wedellborgs Foundation, The Beckett Foundation, The Hede Nielsen Family Foundation, The Dagmar Marshalls Foundation and Manufacturer Einar Willumsen's Memorial Scholarship. These abovementioned funders have had no influence on the study design, will have no influence on collection, management, analysis and interpretation of data, writing of the report or the decision to submit the report for publication. All these activities are done by the authors. Pharma Nord provided the melatonin and placebo tablets but had no influence on the study design and will have no influence on the interpretation of results.

Contributorship Statement

All authors have participated in making substantial contributions to conception and design, drafting the article or revising it critically for important intellectual content and all authors have approved the final version to be published.

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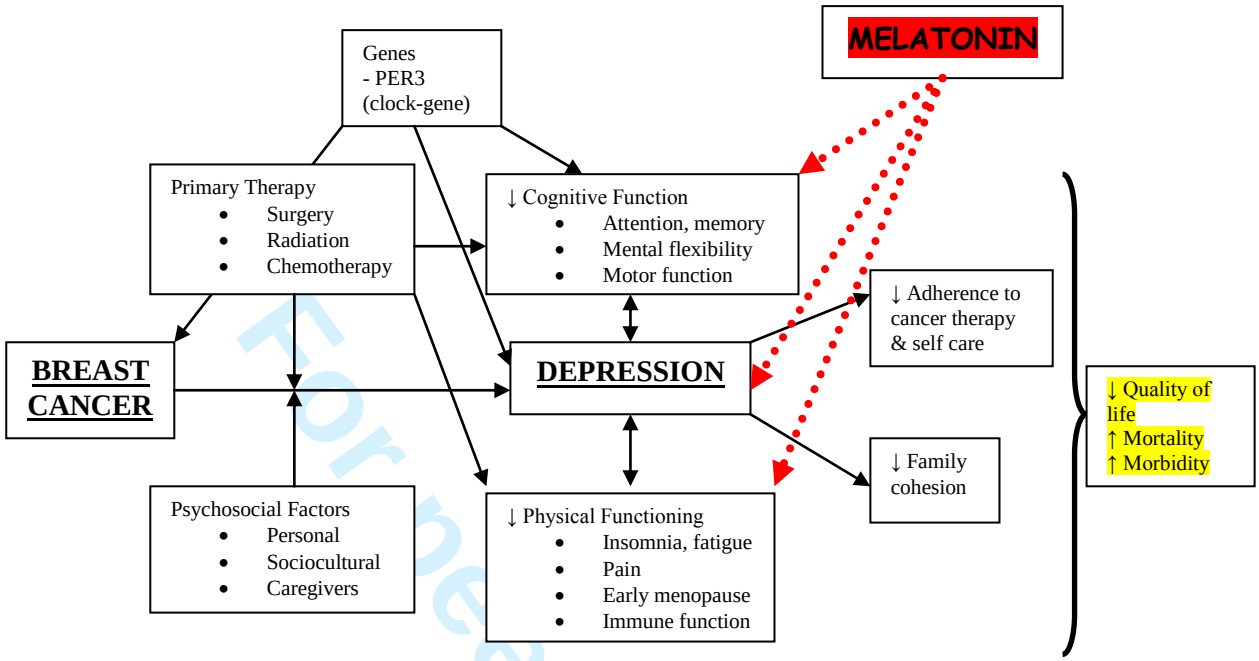
Figure 1: The relationship between breast cancer and depression



Modified after Fann JR et al. 2008 [6]

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3-5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	3-4
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4,8-10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	10
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			5
Sequence generation	8a	Method used to generate the random allocation sequence	
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5-6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5-6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5-6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	3

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	10-11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10-11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	
Recruitment	14a	Dates defining the periods of recruitment and follow-up	
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	1-2
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Statement re. Clinical trial

For the clinical trial

The effect of Melatonin on depression, anxiety, cognitive function and sleep disturbances in breast cancer patients,
ref no. MVH–03–MELODY
Eudract CT no 2010 -022460-12

conducted by

Melissa Voigt Hansen
Department of Surgical Gastroenterology
Herlev Hospital
Denmark

we hereby declare that the test preparations produced for the trial consists of verum and placebo tablets that has the exact similar physical appearance and cannot be distinguished by other means than analysis.

Christian Dan Sindberg, M. Sc.
Pharma Nord Research,
Vejle, December 13th 2011

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Dep. QA/QC	Pharma Nord	Doc. no. L373-00-000
Prep. by K. Frederiksen		Ed nr. 1
Date 160611 / Last ed. ----	Certificate of Analysis	
Auth. by A.Rishede	Product Bio-Melatonin 3 mg v. 1.2	Package Klinisk forsøg blistre

Product code: 373

Batch no.: 1101654

Bulk batch no.: 019860

Date of Manufacture: 07.2010

Expiry Date: 07.2013

Storage: Room temperature.

Test/Assay:	**Results:**	**Limits:**
Appearance:	conforms	Clear coated white round biconvex tablet.
Size:	conforms	7.5 mm
Average weight:	143 mg	137.2-159.4 mg
Uniformity of mass:	conforms	Ph.Eur.
Mass Variation	conforms	Ph.Eur.
Disintegration:	1 min.	< 30 min, Ph.Eur.
Dissolution:	conforms	Q=75 % (45 min)
Identification:		
- Melatonin:	conforms	Conforms to standard, IR
- Melatonin:	conforms	Positive, HPLC
Assay:		
- Melatonin:	3.06 mg	2.85-3.15 mg, HPLC
Impurities:		
-NASE:	0.00 %	≤ 0.5 %
-SERO:	0.00 %	≤ 0.5 %
-DASE:	0.00 %	≤ 0.5 %
-MEXA:	0.00 %	≤ 0.5 %
-Each unknown:	0.00 %	≤ 0.5 %
-Total impurities:	0.00 %	≤ 0.5 %
Microbiological standard *:		
-Total viable aerobic count:	< 100 /g	< 10^3 / g
-Fungi:	< 10 /g	< 10^2 / g
-E-coli:	neg. in 1 g	Neg. in 1 g

*: Assayed once a year. n.a not assayed.

It is hereby certified that the above batch is manufactured, controlled and released according to the principles of Good Manufacturing Practice.

160611 A.Rishede

QA-Department
Pharma Nord ApS, Vojens, Denmark

Dep. QA/QC	Pharma Nord	Doc. no. P373-90-000
Prep. by K. Frederiksen		Ed nr. 1
Date 160611 / Last ed. ----	Certificate of Analysis	
Auth. by A.Rishede	Product Bio-Melatonin 3 mg v. 1.2 Placebo	Package Klinisk forsøg blistre

Product code: 373 (Placebo)

Batch no.: 1101655

Bulk batch no.: 116360

Date of Manufacture: 07.2010

Expiry Date: 07.2013

Storage: Room temperature.

Test/Assay:	**Results:**	**Limits:**
Appearance:	conforms	Clear coated white round biconvex tablet.
Size:	conforms	7.5 mm
Average weight:	143 mg	137.2-159.4 mg
Disintegration:	5 min.	< 30 min, Ph.Eur.
Assay*:		
- Melatonin:	N.d	Not detected
Microbiological standard :		
-Total viable aerobic count:	< 100 /g	< 10^3 / g
-Fungi:	< 10 /g	< 10^2 / g
-E-coli:	neg. in 1 g	Neg. in 1 g

*: N. d not detected.

It is hereby certified that the above batch is manufactured, controlled and released according to the principles of Good Manufacturing Practice.

160611

QA-Department
Pharma Nord ApS, Vojens, Denmark