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UNIT OF NEUROPHYSIOLOGY

CIRCADIAN RHYTHM DYSFUNCTION IN ALZHEIMER'S DISEASE: HOW TO DETERMINE CIRCADIAN PHASE AND THE ROLE OF TAYLORED LIGHT THERAPY ON SLEEP AND COGNITION

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PhD dissertation of

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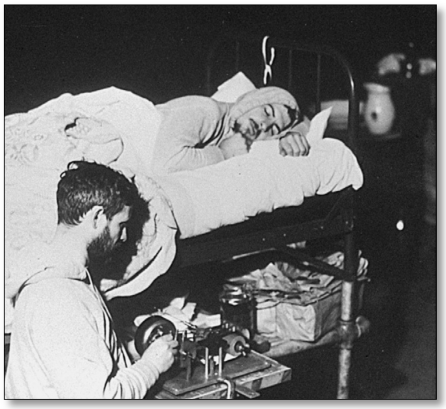

Professor Nathaniel Kleitman,

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SUMMARY

In this thesis circadian rhythm alterations in Alzheimer's disease (AD) are taken into account, in particular concerning the early stage of disease. In the first part of this 3-year work the research was focused on determining the circadian phase in early AD patients using subjective and objective measures: clinical scales, sleep-wake diaries, salivary melatonin determination (DLMO), phase angles between sleep and melatonin onset. To achieved this goal we performed a case-control observational study comparing the circadian phase of early AD patients elderly healthy controls (Hc). The main results of this first step were that Initial evening secretion of melatonin proves to be delayed and mildly impaired in patients with mild/moderate form of Alzheimer disease while patient's subjective sleep parameters and chronotype are reported similar to those of HC. This data indicate that, subclinical altered patterns of melatonin secretion occur in subjects with AD at an early stage of the disease. The second part of the study aimed at comparing circadian phase of early AD patients to the one of another neurodegenerative dementia (OD), determining melatonin secretion in different biological fluids at different daytime. This case-control observational study was performed enrolling patients affected by probable Frontotemporal lobar degeneration (FTLD) and cortico-basal syndrome (CBS) at the early stage of the disease. The main results of this second step were that evening melatonin secretion was confirmed to be delayed and impaired in both AD and OD groups than Hc, but with some important differences between groups: in AD patients melatonin secretion was more delayed and less decreased than OD patients, at this fact was not affected by other factors than disease diagnosis. Furthermore, OD and AD patients showed an higher rate of phase angle alterations and circadian phase misperceptions than Hc. Melatonin secretion proved to correlate in the different biological fluids at a certain time among patients, an this finding support the use of DLMO determination in saliva as a sensitive marker of circadian phase. The third part of the work was focused on the use of a circadian phase modulator in order to interact with the circadian phase of AD patients and evaluate the effect on sleep and cognition. After a systematic review of the literature on this topic, light therapy showed some, albeit limited evidence supporting its use as circadian rhythm modulator in sleep disturbances and agitation in persons with cognitive impairment, with most studies examining light exposure reporting positive effects on at least one sleep measure. However, light

therapy, including BLT, did not show any significant effect in 4 recent systematic reviews and meta-analysis including a Cochrane review specifically examining the results of RCTs. This suggests that the evidence supporting light therapy is at best equivocal due to the heterogeneity in sample size, degree and subtype of dementia, study design, type of light therapy and devices used. The future studies might include randomized controlled trials on specific subtypes of dementia, using preferentially blue-enriched light therapy carefully controlled, which effect must be verified with objective measures. Taking into account this evidences, we performed a single blind randomised control trial to investigate the effects of a tailored light therapy protocol on sleep and cognition parameters in patients with Alzheimer Disease (AD) of mild /moderate severity. The preliminary findings of this study showed that light therapy protocol tailored on the circadian phase proved to be feasible and associated to an objective phase shift in accordance to the melatonin phase response curve, a trend to an increased subjective sleep quality, 24-hour TST and cognitive performance.

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1. INTRODUCTION

1.1 Circadian rhythm alteration in Alzheimer's disease: state of the art

Alzheimer's disease is one of the most debilitating brain disorders among older adults and it is becoming a major public health problem. The prevalence of AD is rapidly increasing and will probably further rise dramatically within the next decades as growing numbers of people are living older.

There is increased evidence suggesting that sleep disorders in neurodegenerative diseases should not be considered an auxiliary disorder, but rather a factor directly related to the neurodegenerative process. Patients with AD often have more fragmented nocturnal sleep than age matched controls, an increase in sleep latency (SL) and a nocturnal total sleep time (TST) reduction[1]. These disturbances are responsible for nocturnal agitation and sleepiness during the day. Alzheimer's disease is also associates to sleep-related disorders, especially Obstructive sleep apnea (OSA) and Restless legs syndrome (RLS)[2]. Sleep disorders worsen as the disease progresses, and their considerable intensification in the late stage of the disease is a strong predictive factor for mortality[3].

A link between sleep characteristics and cognitive decline in the elderly has been repeatedly suggested, emphasizing the fact that sleep and cognition are closely related. Results of clinical and epidemiological studies show a connection between night-time sleep duration and risk of poorer cognitive abilities[4]. Sleep disturbances may in fact exacerbate cognitive symptoms through impairment of sleep-dependent memory consolidation processes, and because of reduced vigilance and impaired attentional functions, such as sustained attention[5].

A relevant role in the genesis of sleep disorders in AD would be played by circadian system dysfunction[6]. Postmortem histological studies in AD reported degeneration of the inner retina with loss of melanopsin retinal ganglion cells (mRGCs) and depletion of related axons in the optic nerve[7]. These findings were more recently confirmed in cohorts of patients studied in vivo with optical coherence tomography showing a thinning of the retinal nerve fiber layer thickness[8], and further supported by the finding of β-amyloid deposits in the retina of AD patients[9]. The mRGCs are intrinsically photosensitive and operate

as photoreceptors influencing circadian rhythms to light/dark cycles through the retinohypothalamic tract[10]. It is known that changes in circadian rhythmicity, traceable by endogenous melatonin dosages, are associated with reduced quality of sleep at night, increased daytime sleepiness and also reduced cognitive performance during the daytime[11,12]. These disturbances can be exacerbated by the placement of patients with AD in controlled environments, where they experience even greater inactivity and reduced exposure to daytime circadian effective light[13].

In the first part of this 3-year work the research was focused on determining the circadian phase in early AD patients using subjective and objective measures: clinical scales, sleep-wake diaries, salivary melatonin determination (DLMO), phase angles between sleep and melatonin onset. To achieved this goal we performed a case-control observational study comparing the circadian phase of early AD patients elderly healthy controls (Hc).

2. STUDY DESIGN

2.1 First step: evaluation of evening melatonin timing secretion in real life conditions in patients with Alzheimer disease of mild to moderate severity

2.1.1.Rationale

This study is aimed at determining dim light melatonin onset (DLMO) in non-institutionalized AD patients investigated by means of an in home salivary melatonin test.

2.1.2 Material and methods

2.1.2.1 Participants selection

The patients were consecutively enrolled at Alzheimer's Disease Assessment Unit of the IRCCS C. Mondino Foundation of Pavia within October 2016 and march 2017 among AD outpatients with a diagnosis of AD according to the standard criteria [14]. The patients were enrolled provided they screened for the following exclusion criteria: score less than 14 or more than 24 at Mini-Mental State Examination (MMSE) [15], visual deficits and glaucoma, diabetes, renal, hepatic or thyroid diseases, abuse of alcohol or substances, use of hypnotics, use of antidepressants, long distance trans-meridian flight(s) in the previous 3 months, intake of melatonin or drugs altering endogenous melatonin secretion, bipolar disorders, seasonal affective disorder or major depression diagnosed according to DSM V criteria [16] and a score more than 13 at Beck Inventory II [17], sleep disorders such as sleep apnea, narcolepsy, restless legs syndrome. As for OSA, AD patients and controls with snoring or with other clinical symptoms/signs suspected for OSAS underwent an out-of-center full-night polygraphic testing with cardiorespiratory channel (Embletta X 100, Embla) polygraphic testing with cardio-respiratory channels and they entered the study only if they had AHI less than five.

Twenty-one out of the 30 AD patients screened, accepted to participate into the study, with their demographic and clinical characteristics being as follows: M/F: 11/10; mean age 74.2±5.4 years, range 61-84; mean disease duration 3.4±1.6 years, range 1-6; MMSE range: 14-24, mean 20.39±3.8; ADL range: 4-6

functions preserved, mean 5.6±0.6; IADL range: 4-6 functions preserved, mean 4.2±1.1 ; NPI range: 3-10, mean 5.8±2.2; CDR range: 1-2, mean 1.1±0.6.

All the patients were on treatment with acetylcholinesterase inhibitors (donepezil or rivastigmine); the dosage of donepezil ranging between 5 and 10 mg/day in a single oral daily administration; the dosage of rivastigmine transdermal patch ranging between 4.6 and 9.5 mg.

Healthy subjects (HC) coming from the same geographic area and in the same age range of AD patients (60-85 years) were selected from our database to serve as controls. All the controls had to score more than 24 at Mini-Mental State Examination (MMSE) and to meet the above mentioned exclusion criteria. Seventeen HC could be selected (M/F: 10/7; mean age was 67.47±3.8 years, range 61-78).

No subject was financially compensated. All the participants signed a consent form before entering the study. The protocol was approved by the Ethics Committee of the IRCCS C. Mondino Foundation. This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

The following quantitative instruments were used to describe the AD population:

- Mini Mental State Examination (MMSE) for global cognitive evaluation;
- Activities of Daily Living (ADL), maintained functions were considered [18];
- Instrumental Activities of Daily Living (IADL), maintained functions were considered [19];
- Neuropsychiatric Inventory (NPI) [20];
- Clinical Dementia Rating (CDR) [21] to evaluate disease severity.

2.1.2.2 Procedures

The participants underwent a semi-structured sleep interview and filled in a validated Italian version of the Morningness-Eveningness Questionnaire (MEQ)[22], the Pittsburgh Sleep Quality Index (PSQI)[23] and the Sleep Condition Indicator (SCI)[24] while a physician expert in sleep disorders was available for any clarification and check of the questionnaires. The participants were then instructed to keep a sleep graphic log at home for two weeks maintaining their usual living conditions. On the 15th day, they were instructed to do an in-home salivary melatonin test (Bühlmann Laboratories AG, Schonenbuch, Switzerland) according to the specific, published procedures [25] and described below.

2.1.2.3 Sleep questionnaires

2.1.2.3.1 Semi-structured sleep interview and sleep logs

The aim of the semi-structured sleep interview was to assess the timing and duration of sleep, sleep hygiene, and symptoms of sleep disorders, including daytime sleepiness, snoring, sleep apnea, cataplexy, sleep-related motor disorders and parasomnias.

2.1.2.3.2 Morningness–Eveningness Questionnaire

The MEQ was used to evaluate the subjective/behavioural chronotype. Chronotype was defined according to the standard range of score values: 41 or less “evening type”, 59 and more “morning type”, scores between 42 and 58 “intermediate type”.

2.1.2.3.3 Pittsburgh Sleep Quality Index

The PSQI was used to evaluate the subjective sleep quality, with poor sleep quality being indicated by a score greater than five (maximum score: 21).

2.1.2.3.4 Sleep Condition Indicator.

The Sleep Condition Indicator was used in identifying a condition of insomnia. A global score less than 16 was taken as indicative of the existence of a condition of insomnia.

2.1.2.4 Melatonin test

Melatonin testing was done during a period of daylight saving in april 2017. The participants had to collect five salivary samples in a dark (< 10 lux) environment, at hourly intervals with the first sample collected three hours before their usual bedtime.

Melatonin concentration in salivary samples was measured using a commercial ELISA kit (Direct Saliva MELATONIN– Buhlmann), according to the manufacturer's instructions. Briefly, saliva samples were pre-treated with a pretreatment solution and 100µl of each sample was added to the wells. Each ELISA test included control (low and high) samples.

The optical density at 450 nm was determined using a microplate reader (Biotek). DLMO was calculated by linear interpolation across the time points before and after the melatonin concentration increased to and stayed above 3 pg/mL. DLMO

was expressed in 24-hour clock time and single DLMO values with respect to the circadian phase definition were interpreted according to published range of values indicating that: values before 7:30 pm are indicative of an early circadian phase, values between 7:30 pm–10 pm of an intermediate phase and values after 10 pm of a late circadian phase [26].

To compare the salivary melatonin secretion patterns we aligned each of the five salivary samples according to its temporal relationship to the first saliva sample prior to DLMO. A seven-point curve (three pre- and four post-DLMO) was obtained. The following parameters were taken as measure of the melatonin secretion pattern following DLMO:

- The melatonin salivary concentration of the first post-DLMO melatonin sample (post-DLMO measure) (pg/mL);
- The melatonin secretion rate shortly after DLMO, expressed as the melatonin surge in the 30-minute time interval following DLMO (post-DLMO surge) (pg/[mL*h]);
- The under the curve area of the post-DLMO semicurve (AUC) ([pg*h]/mL);
- The under the curve area specifically about the 30-minute time interval of melatonin secretion after DLMO occurrence (AUC30) ([pg*h]/mL);

2.1.2.5 Outcome measures

2.1.2.5.1 Sleep questionnaire and tests:

MEQ, PSQI and SCI scores.

2.1.2.5.2 Melatonin Test

DLMO was taken as an objective circadian phase marker.

Other measures of melatonin secretion, namely post-DLMO measure, post-DLMO surge, AUC and AUC30, were also taken into consideration in evaluating melatonin secretion patterns.

2.1.2.6 Statistical analysis

To calculate the sample size for this study we referred to the indications of the "Open Source Epidemiologic Statistics for Public Health" (www.openepi.com). Based on literature data and our previous experience [27], we took as meaningful a difference of 45 min in DLMO time between AD patients and HC. The sample size was calculated according to the following parameters: confidence interval

(two-sided) 95%, power 80%, ratio of sample size 1, mean difference 45 min, and standard deviation of 60 min for both groups. The suggested minimum number of subjects to be enrolled was 56 (28 per group). The Statistical Package for the Social Sciences (SPSS) for Windows, version 21.0, was used for the statistical analysis with the normality of distribution of all our variables being assessed in terms of “skewness” and “excess kurtosis”. All data proved to have a Gaussian distribution. Categorical variables were submitted to cross-tabulation, and statistical significance was evaluated using the chi-square test or Fisher's exact test, where appropriate. As far as continuous variables were concerned, they were expressed as mean values ± standard deviation and differences between groups were tested with ANOVA (analysis of variance) followed by a post hoc Bonferroni test. The level of significance was set at 0.05. AD patients and HC differ significantly for age with patients being older. Since age may influence sleep as well as melatonin parameters, any significant difference between AD patients and HC at univariate/bivariate analyses, was further tested in a linear regression analysis.

2.1.3 Results

Sleep measures and subjective measures of chronotype are detailed in ***table 1***.

		Alzheimer Disease Patients	Healthy Controls		p-value
Age		74.14±5.4	67.47±3.8		**<u>0.001</u>**
Male Sex (%)		11 (52.4%)	10 (58.8%)		0.691
MEQ mean values		61.14±7.9	56.88±8.3		0.116
MEQ Categorial values	Morningness	11 (52.4%)	7 (41.2%)		0.464
	Intermediate	10 (47.6%)	9 (52.9%)		
	Eveningness	0 (0.0%)	1 (5.9%)		
PITTSBURGH SCORE		6.24±5.4	4.47±3.0		0.237
PITTSBURGH SCORE> 5		8 (38.1%)	5 (29.4%)		0.575
SCI		26.29±9.1	25.45±7.3		0.800
SCI < 16		2 (11.8%)	1 (9.1%)		0.823

Table 1- *Sleep parameters and subjective chronotypes in AD patients and HC.*

2.1.3.1 Sleep measures

Subjective sleep quality did not differ between groups. In fact, the percentage of subjects with a PSQI score above 5 did not differ between patients and controls (AD: 38.1%, HC: 29.4%, p = 0.57) and neither did the mean PSQI score (AD: 6.24 ± 5.4, HC: 4.47 ± 3.0, p= 0.24). AD patients used to go to bed earlier with respect to HC both on workdays (AD 22:42±00:56 vs HC 23:51±00:27) and free-days (AD 22:44±00:58 vs HC 23:58±00:58), with the difference being statistically significant (p < 0.01, ***figure 1***).

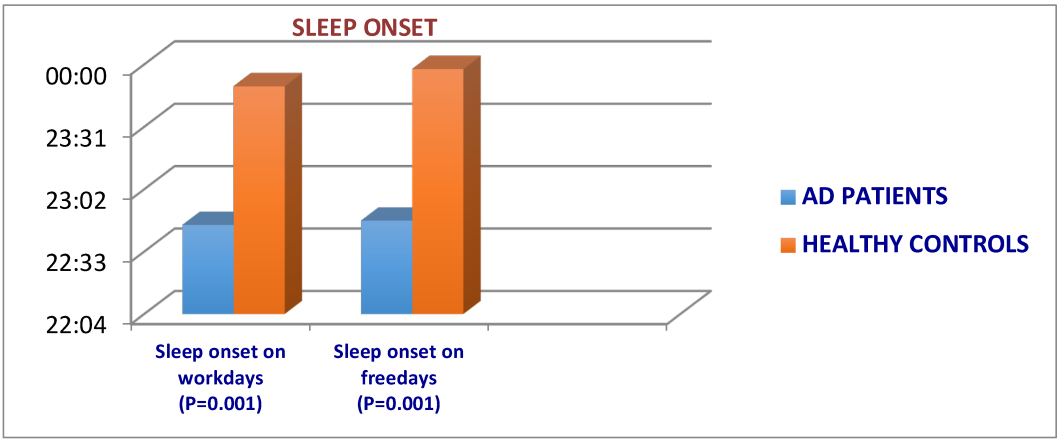

Figure 1- *Sleep onset mean values expressed in daytime in groups.*

As for SCI, the mean SCI score did not differ significantly between AD patients and HC (26.29 +/- 9.1vs 25.45+/-7.3; p=0.08). The percentage of subjects with a SCI score < 16 did not differ between AD patients and HC (11.8% versus 9.1%; p=0.823).

2.1.3.2 Subjective measures of chronotype

The mean MEQ score (61.14±7.9 versus 61.14±7.9, p = 0.116) as well as the subjective chronotype distribution by global MEQ score did not differ between Ad patients and HC.

2.1.3.3 Melatonin measurements

We failed to calculate DLMO in five patients. In one patient because the quantity of saliva sampling was insufficient to allow a correct melatonin determination, in two patients because the melatonin concentration was constantly below the

salivary melatonin threshold of 3 pg and in other two patients because the melatonin concentration proved to be either far higher than the a salivary melatonin threshold of 3 pg or showed bizarre fluctuations. Thus DLMO could be definitely calculated in 17 out of the 21 patients examined (86.6%) while it could be calculated in all the controls.

Dim light melatonin onset time and melatonin secretion parameters are detailed in ***table 2.***

		Alzheimer Disease Patients	Healthy Controls		p-value
Age		74.14±5.4	67.47±3.8		**0.001**
Male Sex (%)		11 (52.4%)	10 (58.8%)		0.691
DLMO		22:04±01:31	21:09±00:40		**0.028**
Chronotype according to DLMO	Matt.	0 (0%)	0 (0%)		**0.019**
	Int.	9 (52.9%)	16 (88.9%)		
	Ser.	8 (47.1%)	2 (11.1%)		
AUC-1		1.78±0.7	2.90±0.8		**0.002**
AUC-2		3.19±1.2	5.38±1.9		**0.001**
AUC-3		5.52±2.7	8.5±3.4		**0.012**
AUC-4		6.04±3.0	11.71±5.2		**0.009**
AUC-TOTAL		11.75±6.12	26.11±10.1		**0.001**
AUC-Theoric		4.46±1.4	4.92±1.58		0.386
MELATONIN-1		1.76±0.7	2.14±0.7		0.162
MELATONIN-2		4.70±2.3	4.62±1.2		0.901
MELATONIN-3		6.10±3.8	9.12±6.2		0.107
MELATONIN-4		6.60±3.4	11.09±6.4		0.096
SECRETION		2.92±2.7	3.84±3.2		0.386

Table 2- *Melatonin secretion parameters in AD patients and HC.*

On average DLMO occurred significantly later in AD patients than in controls as displayed in ***figure 2*** (55 minutes; p=0.028), with its single values falling within the range of DLMO values indicative of evening chronotype in AD patients and of an intermediate-type in controls [25].

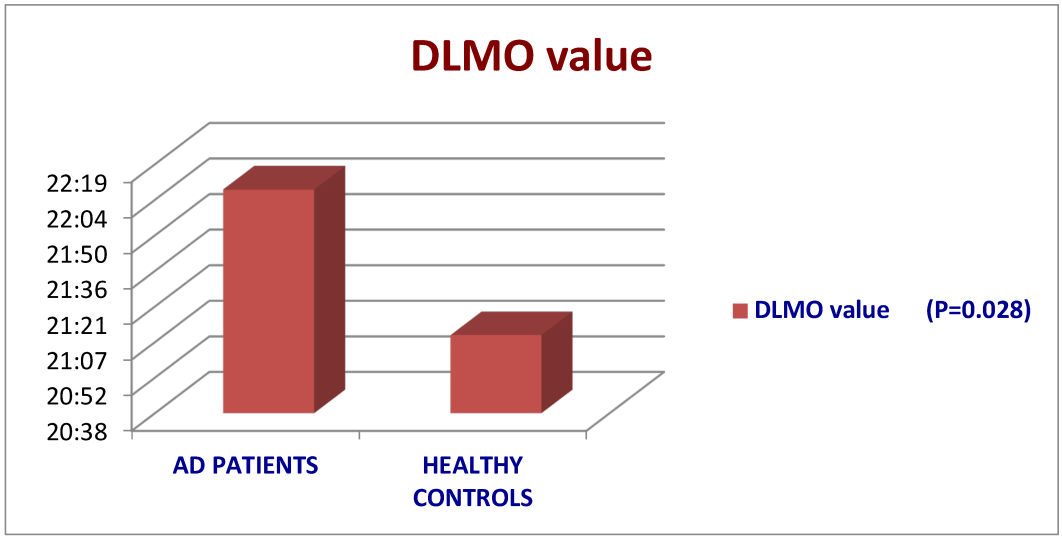

Figure 2- *Mean DLMO values expressed in daytime in both groups.*

Analysis of the semicurve of salivary melatonin secretion before and after DLMO showed that post-DLMO melatonin measures were significantly lower in AD patients *(**figure 3**)*.

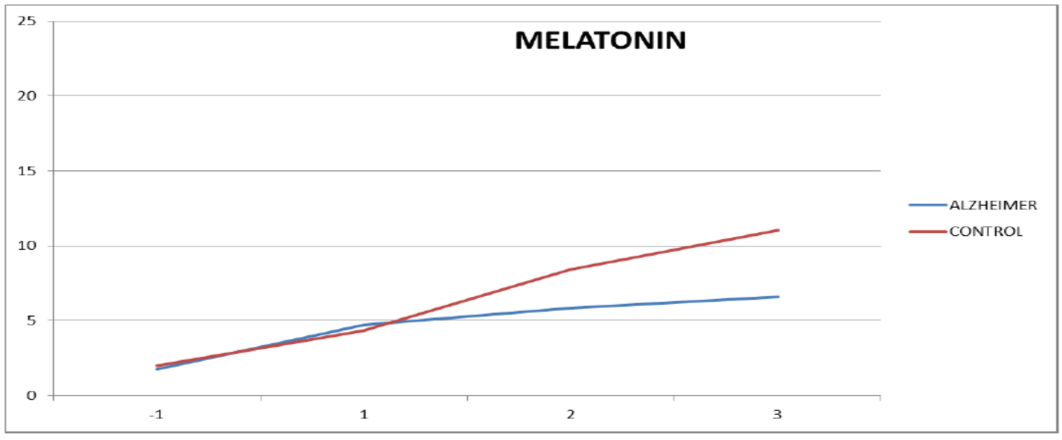

Figure 3 *- Melatonin secretion curve in AD patients (blue line) and controls (red line). The X axis represents 60- minute consecutive intervals in relation to DLMO (pre and post-DLMO). The Y axis represents the melatonin concentration in saliva at each point of determination.*

This result was confirmed by evaluating the single and global melatonin AUC semicurve between groups (***figure 4***; p=0.001). The linear regression analysis showed that DLMO was independent from age, while it was statistically related to AD diagnosis (Age, beta: -0.146 , p= 0.501; AD patients/ HC , beta: -0.466, p= 0.038, R=0.387).

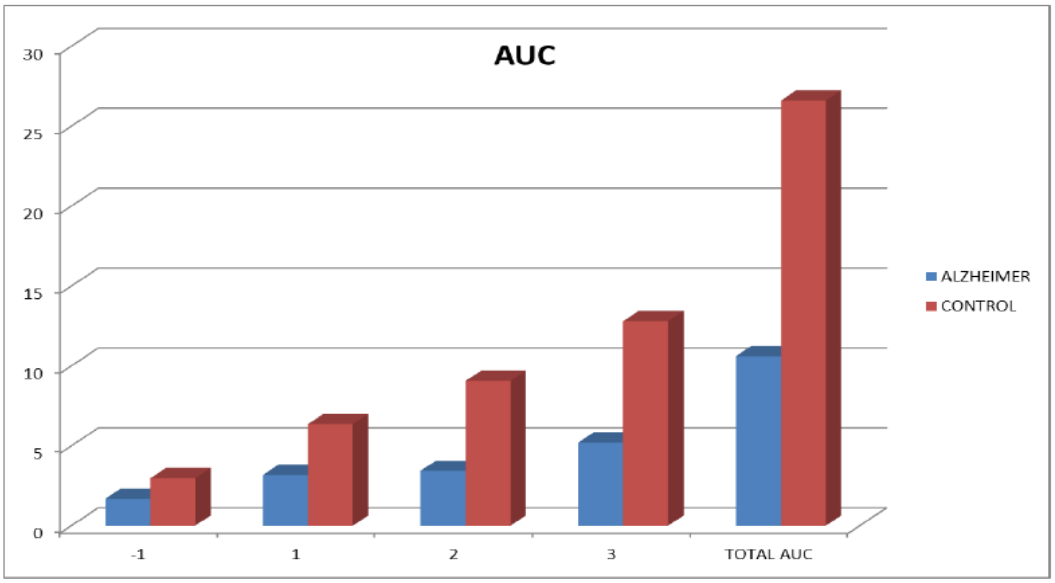

Figure 4- *Under-the-curve area of the melatonin secretion curves (AUC) in relation to DLMO (pre and post-DLMO) in AD patients (blue) and controls (red). (p= 0.001). X axis represents 60- minute consecutive intervals in relation to DLMO. The Y axis represents the melatonin concentration in saliva at each point of determination.*

2.1.3.4 Sleep parameters and melatonin measurements according to disease severity

Patients with a global MMSE score >18, indicating a mild cognitive impairment, were compared to those with a global MMSE score ≤ 18. Neither differences in subjective and objective sleep parameters, nor in DLMO and melatonin secretion pattern were found (MEQ mean score p=0.827; Pittsburgh score p=0.645; SCI p=0.179; DLMO p=0.698; AUC p=0.605). No correlation was found between melatonin measurements and NPI.

2.1.4 Discussion

The few literature data concerning melatonin secretory patterns in AD refer to in-hospital or community- dwelling, with overt sleep disturbances AD patients investigated while kept in standardized conditions. Irregular, variable patterns of reduced melatonin secretion were reported in 24-hour plasma level melatonin determinations in such AD patients with respect to regular nictemeral patterns in controls[28-29]. We investigated secretory patterns of endogenous melatonin in patients with AD of mild to moderate degree and without comorbidities.

Our patients reported mild sleep or no sleep complaints at odd with high prevalence of sleep disorders reported in AD in the literature[30]. This discrepancy is likely due to the fact that literature data refers to samples including AD patients with comorbidities and high degree of disease severity, while our data pertains to AD patients of mild to moderate degree of severity and without comorbidities, such as depression and sleep apnea.

In investigating endogenous secretive melatonin patterns we focused on the timing of melatonin secretion by determining DLMO according to standard melatonin salivary testing. Our data indicate that DLMO tends to occur on average an hour later in AD patients than in HC. In spite of this, AD patients tend to go to bed earlier than HC do and anyway early with respect to their DLMO clock time. This is likely due to their withdrawal from social and family activities with a tendency to isolation. To go to bed early with respect to DLMO clock time was reported to play a role in causing insomnia[31]. The discrepancy between bedtime and DLMO clock time in AD could be a potential determinant of overtime insomnia development in these patients.

Pre and post DLMO values of secretion show that melatonin secretion is mildly decreased in our AD patients with respect to HC in keeping with literature data documenting globally decreased melatonin secretion in AD[32-28]. It is known that circadian phase tends to physiologically advance with aging as documented in studies comparing peak time of plasma melatonin in healthy young and old people investigated in constant routine as well as in standardized daily routine conditions[33]. By contrast we found a trend to a delay of the circadian phase in our AD patients in spite they were older than HC. Our findings are in keeping with studies documenting delayed rest-activity actigraphic patterns [34] and delayed core body temperature in AD [35].

Circadian phase shifting is reportedly associated with cognitive decline progression in older adults [31], disordered sleep and sleep-related abnormal behavior such as sundown in advanced forms of AD [36].

Our data indicate that altered melatonin secretion, may occur in AD patients at an early stage of the disease and in lack of overt sleep complaints. In fact, based on subjective data, AD patients show sleep quality similar to that of HC, with only one third of the patients reporting mildly poor sleep quality. Accordingly the frequency of insomnia disorder as assessed by the SCI is low in our AD patients and not statistically different compared to that of HC.

MEQ scores are similar in patients and controls and indicate an intermediate subjective chronotype. Albeit within the limits of AD patients potential misjudgment due to cognitive deficits and anosognosia [37], the data indicate a quite preserved sleep quality and neutral subjective chronotype in our AD patients. So the melatonin secretion alterations we found would represent an early sign of melatonin secretion dysregulation in AD. Further and longitudinal data are warranted to confirm the present findings and to clarify their ultimate neurobiological meaning and clinical importance in AD. These data could pave the way to new therapeutic strategies in AD by exploiting the chronobiotic properties of melatonin in resetting circadian rhythms.

Our paper suffers from some limitations. A 7-day actigraphic monitoring would have provided more reliable evaluation of sleep-wake patterns in patients and controls. Even though instrumental monitoring is not required during in-home melatonin salivary test, actigraphic monitoring along with light sensor would have provided reliable measures of rest activity patterns of the patients and of their compliance with respect to stay in a dark environment while performing the test.

2.2 Second step: circadian phase comparison in Alzheimer's disease and other degenerative dementias at the early stage of disease

This second part of the study aimed at comparing circadian phases of early AD patients and another neurodegenerative dementia (OD), determining melatonin secretion in different biological fluids at different daytime. This case-control observational study was performed enrolling patients affected by probable Frontotemporal lobar degeneration (FTLD) and Cortico-basal syndrome (CBS) at the early stage of the disease.

2.2.1 Rationale

The main aim of this study is to determine and compare the circadian phase (DLMO) of AD patients and another neurodegenerative dementia (FTLD/CBS) at the early stage of disease. A secondary aim concerns the measures of melatonin secretion in different biological fluids (saliva, blood, CSF,urine) at different daytime.

2.2.2 Material and methods

2.2.2.1 Participants selection

Patients affected by probable Alzheimer Disease, Fronto-temporal lobar degeneration and Cortico-basal syndrome were consecutively enrolled at Neurology-Neurorehabilitation Unit of the IRCCS Istituto Auxologico Piancavallo within October 2017 and march 2019 among outpatients with a diagnosis of cognitive impairment according to the standard criteria including CSF biomarkers of neurodegeneration [38]. The patients were enrolled provided they screened for the following exclusion criteria: score less than 14 or more than 27 at Mini-Mental State Examination (MMSE) [15], visual deficits and glaucoma, diabetes, renal, hepatic or thyroid diseases, abuse of alcohol or substances, use of hypnotics, use of antidepressants, long distance trans-meridian flight(s) in the previous 3 months, intake of melatonin or drugs altering endogenous melatonin secretion, bipolar disorders, seasonal affective disorder or major depression diagnosed according to DSM V criteria [16] and a score more than 13 at Beck Inventory II [17], sleep disorders such as sleep apnea, narcolepsy, restless legs syndrome. As for OSA, AD patients and controls with snoring or with other clinical

symptoms/signs suspected for OSAS underwent an out-of-center full-night polygraphic testing with cardiorespiratory channel (Embletta X 100, Embla) polygraphic testing with cardio-respiratory channels and they entered the study only if they had AHI less than five.

Five out of 8 AD patients and eighteen out of the 25 other neurodegenerative patients (OD; FTLD/CBS) screened, accepted to participate into the study. Thirty-six AD patients and 21 healthy controls previously enrolled in step one study served as comparing groups.

The demographic and clinical characteristics of the three groups have been summarized in ***table 3.***

Patients group	**Sample size** (n)	**Sex** (male/ female)	**Age** (years, mean ±sd)	**Disease severity** (MMSEc, mean ±sd)	**Disease severity** (n MCI/dementia)	**Disease duration** (months, mean ±sd)
Alzheimer Disease	41	21 M/20 F	73,34 ± 5,65	20,96 ± 3,73	9 MCI/32 dem	29,80 ± 19,78
Other Neurodegenerative Dementia (FTLD,CBS)	18	5 M/3 F	73,49 ± 6,77	23,68 ± 4, 67	14 MCI/4 dem	29,44 ± 27,4
Healthy controls	21	12M/9F	67,29 ± 4,05			

Table 3- *Demographic and clinical characteristics of groups*

All AD patients were on treatment with acetylcholinesterase inhibitors (donepezil or rivastigmine); the dosage of donepezil ranging between 5 and 10 mg/day in a single oral daily administration; the dosage of rivastigmine transdermal patch ranging between 4.6 and 9.5 mg.

No subject was financially compensated. All the participants signed a consent form before entering the study. The protocol was approved by Ethics Committees of the IRCCS C. Mondino Foundation and Istituto Auxologico Piancavalo. This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

The following quantitative instruments were used to describe the AD and OD population:

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- Clinical Dementia Rating (CDR) [21] to evaluate disease severity.

2.2.2.2 Procedures

The participants underwent a semi-structured sleep interview and filled in a validated Italian version of the Morningness-Eveningness Questionnaire (MEQ) [22], the Pittsburgh Sleep Quality Index (PSQI) [23], Epworth sleepiness scale [39] and the Sleep Condition Indicator (SCI) [24] while a physician expert in sleep disorders was available for any clarification and check of the questionnaires.

The participants enrolled at IRCCS Mondino Institute were instructed to keep a sleep graphic log at home for two weeks maintaining their usual living conditions. On the 15th day, they were instructed to do an in-home salivary melatonin test (Bühlmann Laboratories AG, Schonenbuch, Switzerland) according to the specific, published procedures [25] and described below.

For participants enrolled at IRCCS Istituto Auxologico Piancavallo, the same salivary melatonin test was performed in hospital setting under supervision of a sleep medicine expert. Patients were provided with sun glasses and ear plugs to protect themselves from environmental interferences during the test. The day after this test, in the morning time at 8.00 a.m. patients underwent a single spot salivary melatonin test , a blood sample and a CSF sample for melatonin concentration measure in these fluids. Urinary melatonin metabolite was also determined by 24 hour urine collection starting from 11. a.m. the same day of salivary melatonin test.

2.2.2.3 Sleep questionnaires

2.2.2.3.1 Semi-structured sleep interview and sleep logs

The aim of the semi-structured sleep interview was to assess the timing and duration of sleep, sleep hygiene, and symptoms of sleep disorders, including daytime sleepiness, snoring, sleep apnea, cataplexy, sleep-related motor disorders and parasomnias.

2.2.2.3.2 Morningness–Eveningness Questionnaire

The MEQ was used to evaluate the subjective/behavioural chronotype. Chronotype was defined according to the standard range of score values: 41 or less “evening type”, 59 and more “morning type”, scores between 42 and 58 “intermediate type”.

2.2.2.3.3 Pittsburgh Sleep Quality Index

The PSQI was used to evaluate the subjective sleep quality, with poor sleep quality being indicated by a score greater than five (maximum score: 21).

2.2.2.3.4 Sleep Condition Indicator.

The Sleep Condition Indicator was used in identifying a condition of insomnia. A global score less than 16 was taken as indicative of the existence of a condition of insomnia.

2.2.2.3.5 Epworth sleepiness scale

The Epworth sleep scale was used to evaluate the subjective sleep somnolence, with higher sleepiness being indicated by a score greater than ten (maximum score: 24).

2.2.2.4 Melatonin test

Concerning the evening melatonin salivary determination, the participants had to collect five salivary samples in a dark (< 10 lux) environment, at hourly intervals with the first sample collected three hours before their usual bedtime.

Melatonin concentration in salivary samples was measured using a commercial ELISA kit (Direct Saliva MELATONIN– Buhlmann), according to the manufacturer's instructions. Briefly, saliva samples were pre-treated with a pretreatment solution and 100µl of each sample was added to the wells. Each ELISA test included control (low and high) samples.

The optical density at 450 nm was determined using a microplate reader (Biotek). DLMO was calculated by linear interpolation across the time points before and after the melatonin concentration increased to and stayed above 3 pg/mL. DLMO was expressed in 24-hour clock time and single DLMO values with respect to the circadian phase definition were interpreted according to published range of values indicating that: values before 7:30 pm are indicative of an early circadian

phase, values between 7:30 pm–10 pm of an intermediate phase and values after 10 pm of a late circadian phase [26].

DLMO timing was also related to bedtime to determine the phase angle DLMO-bedtime. Bedtime was considered the time of the night patients indicated to fall asleep. A phase angle DLMO-bedtime ≥ 1 hour indicates a bedtime right circadian phase (BTRCP), while a value < 1 hour indicates a bedtime wrong circadian phase (BTWCP), according to literature data [40].

To compare the evening salivary melatonin secretion patterns we evaluated:

- The under the curve area of the five points DLMO semicurve (AUC) ([pg*h]/mL);
- The under the curve area specifically about the 30-minute time interval of melatonin secretion after DLMO occurrence (AUC30) ([pg*h]/mL);

The day after the evening salivary DLMO test, in the morning time at 8.00 a.m. patients underwent a single spot salivary melatonin determination, a blood sample and a CSF sample for melatonin concentration measure in these fluids. Urinary melatonin metabolite was also determined by 24 hour urine collection starting from 11.00 a.m. the same day of salivary melatonin test.

2.2.2.5 Outcome measures

2.2.2.5.1 Sleep questionnaire and tests:

MEQ, PSQI, ESS and SCI scores.

2.2.2.5.2 Melatonin Test

DLMO was taken as an objective circadian phase marker.

Other melatonin secretion related behavioural parameters, namely BTWCP, evening AUC and AUC30, morning melatonin plasma measure, morning melatonin CSF measure, 24 hour urinary metabolite melatonin measure were also taken into consideration. However, the 24 h urinary melatonin metabolite concentration was not taken into account because of bias during sample collection.

2.2.2.6 Statistical analysis

To calculate the sample size for this study we referred to the indications of the “Open Source Epidemiologic Statistics for Public Health” (www.openepi.com). Based on literature data and our previous experience [27], we took as meaningful a difference of 45 min in DLMO time between AD or OD patients and HC. The

sample size was calculated according to the following parameters: confidence interval (two-sided) 95%, power 80%, ratio of sample size 1, mean difference 45 min, and standard deviation of 60 min for both groups. The suggested minimum number of subjects to be enrolled was 84 (28 per group). The Statistical Package for the Social Sciences (SPSS) for Windows, version 21.0, was used for the statistical analysis with the normality of distribution of all our variables being assessed in terms of “skewness” and “excess kurtosis”. All data proved to have a Gaussian distribution. Categorical variables were submitted to cross-tabulation, and statistical significance was evaluated using the chi-square test or Fisher's exact test, where appropriate. As far as continuous variables were concerned, they were expressed as mean values ± standard deviation and differences between groups were tested with ANOVA (analysis of variance) followed by a post hoc Bonferroni test. The level of significance was set at 0.05. AD patients and HC differ significantly for age with patients being older. Since age may influence sleep as well as melatonin parameters, any significant difference between AD patients and HC at univariate/bivariate analyses, was further tested in a linear regression analysis.

2.2.3 Results

AD patients showed a mean DLMO value delayed compared to both OD patients and healthy controls (AD DLMO 21:32 ± 1:41, OD DLMO 21:11 ± 1:40, Hc 21:09 ± 0:39). Interestingly, focusing on disease severity, MCI AD patients had a markedly delayed circadian phase compared to MCI OD (MCI AD DLMO 22:21 ± 1:37, MCI OD DLMO 20:52 ± 1:35), whereas AD dementia patients showed a DLMO value comparable to healthy controls (AD dementia DLMO 21:17 ± 1:39). The percentage of low secretors did not differ between groups and subgroups.

Categorizing DLMO values in accordance with literature data, late circadian phase subtype (DLMO>22.00 h) was significantly higher in AD and OD patients compared to controls (***Figure 5***; p=0.002).

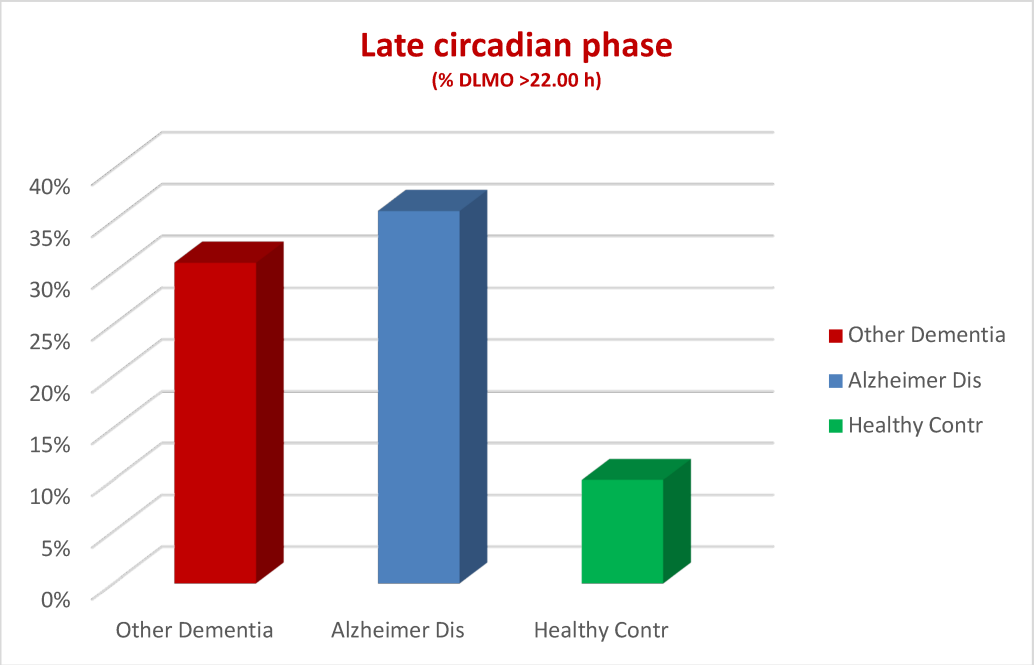

Figure 5- *Late circadian phase distribution between groups.*

When DLM0 was evaluated with respect to bedtime, a higher percentage of Bedtime-DLMO wrong circadian phase (BTWCP) was found in both OD and AD groups compared to controls (***Figure 6***; p=0.003).

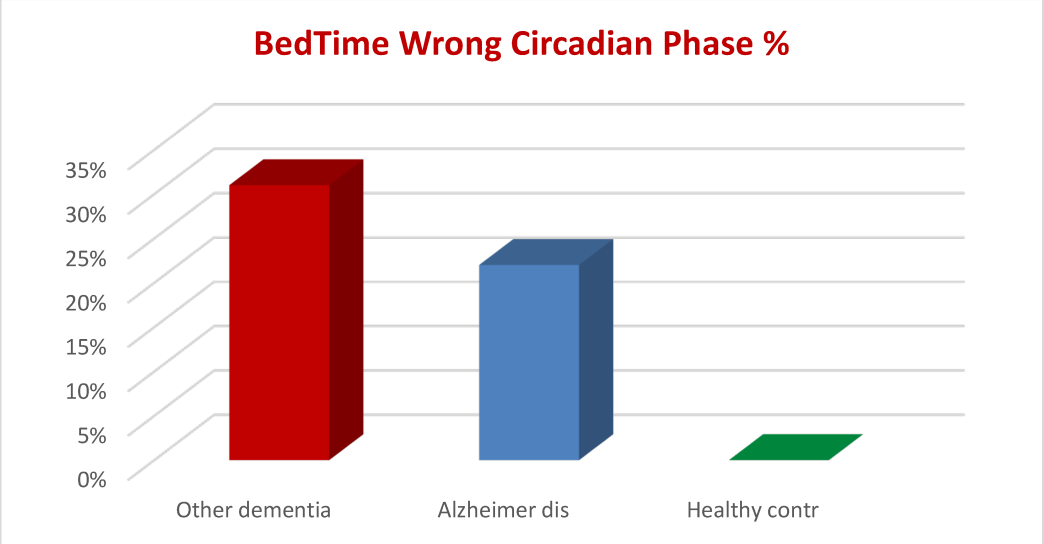

Figure 6- *BTWCP distribution between groups.*

Comparing subjective chronotype (MEQ score) to the circadian phase (DLMO), both OD and AD groups proved to present a higher rate of patients with an evening circadian phase than what expected taking into account patients' usual subjective chronotype (***Figure 7***; p=0,003).

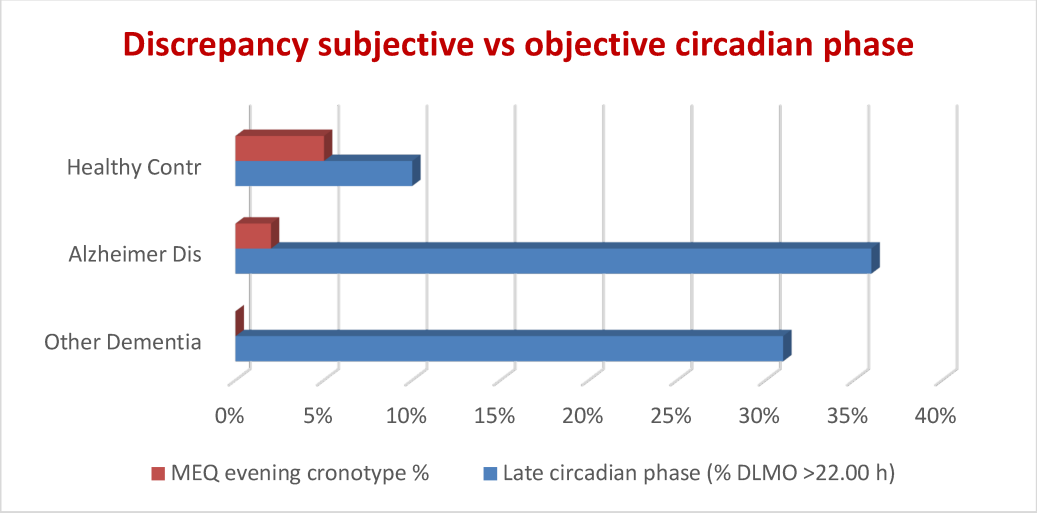

Figure 7- *Comparison between MEQ late chronotype and DLMO late circadian phase distribution between groups.*

Furthermore, evening melatonin secretion total AUC was deeply decreased in OD group compared to AD and Hc, and was still significantly decreased in AD compared to Hc (***Figure 8***; p=0.00008).

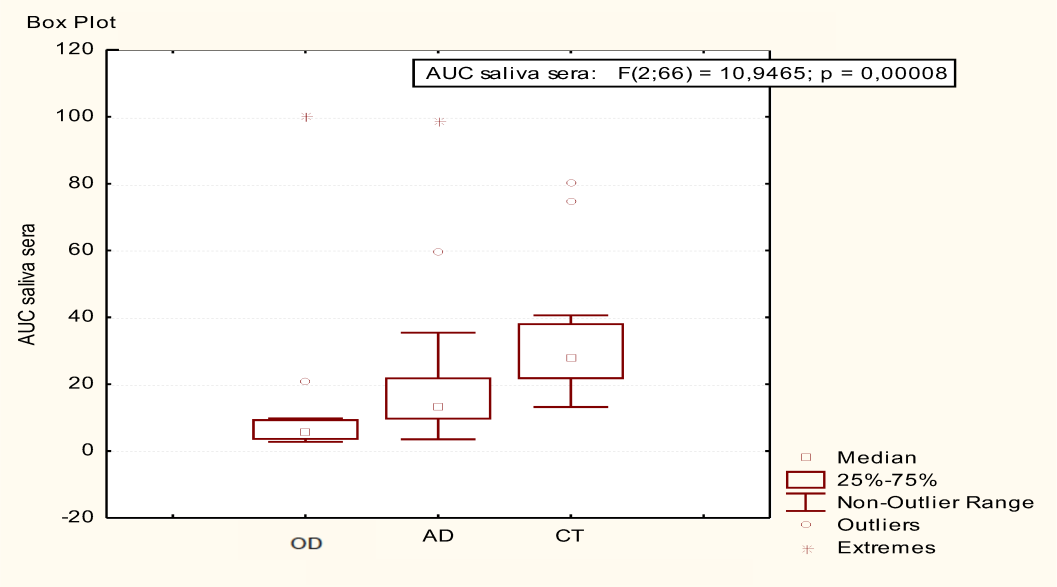

Figure 8- *Box plot showing AUC distribution between groups*

When AUC had been normalized for DLMO value, taking into account the first 30 minutes secretion post DLMO (AUC 30), AD group showed values comparable to Hc, while OD group confirmed a significant decrease in the evening melatonin secretion (***Figure 9***; p=0,0002). The discrepancy in AD group between AUC and AUC 30 suggests the presence of a delay but not a reduction in evening melatonin secretion. Conversely, in OD group a significant reduction in evening melatonin secretion was confirmed by AUC 30 values.

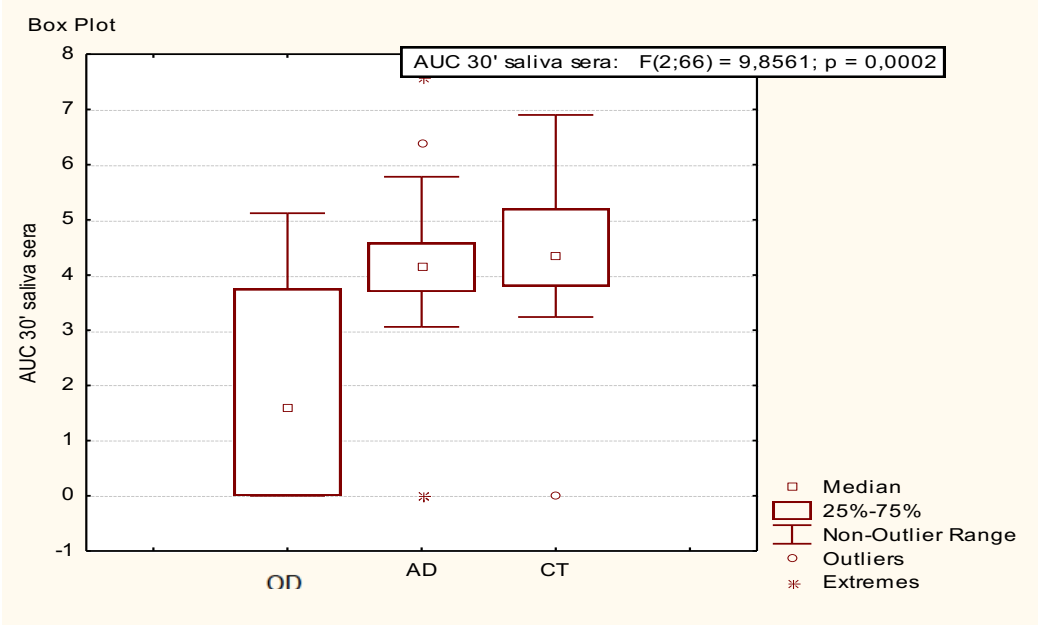

Figure 9- *Box plot showing AUC30 distribution between groups*

MCI subgroups are difficult to be statistically evaluated because of small sample size, but when Anova has been performed between subgroups, MCI patients follow the same correlations to AUC and AUC 30 with respect to the corresponding demented ones (***Figure 10***, p=0,0005; ***Figure 11***, p= 0,0014).

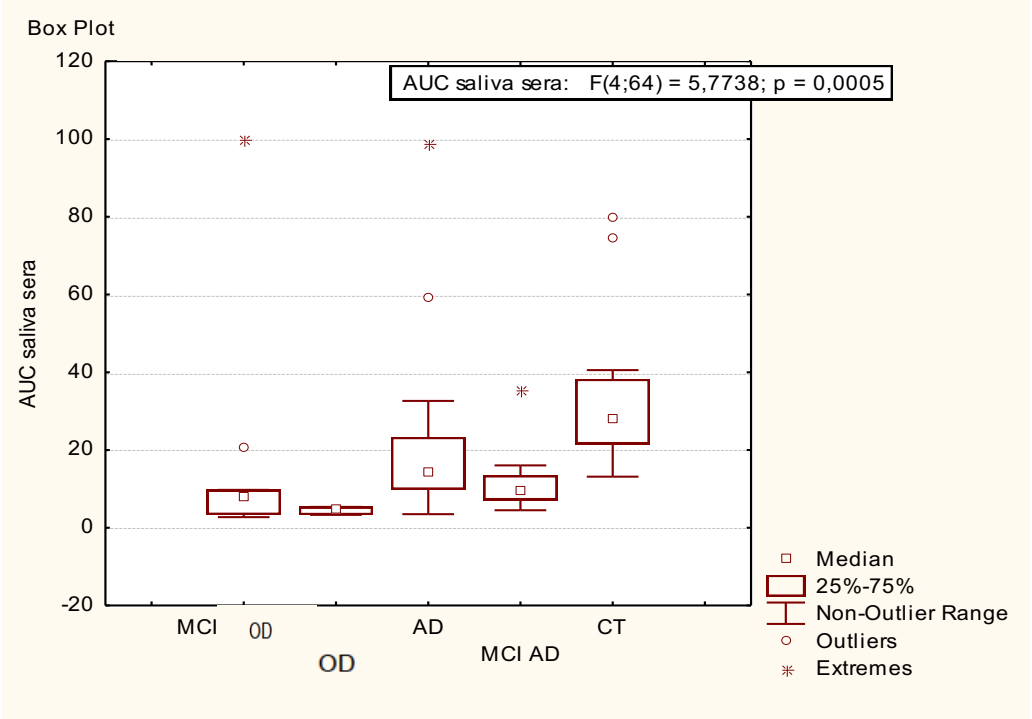

Figure 10- *Box plot showing AUC distribution between subgroups.*

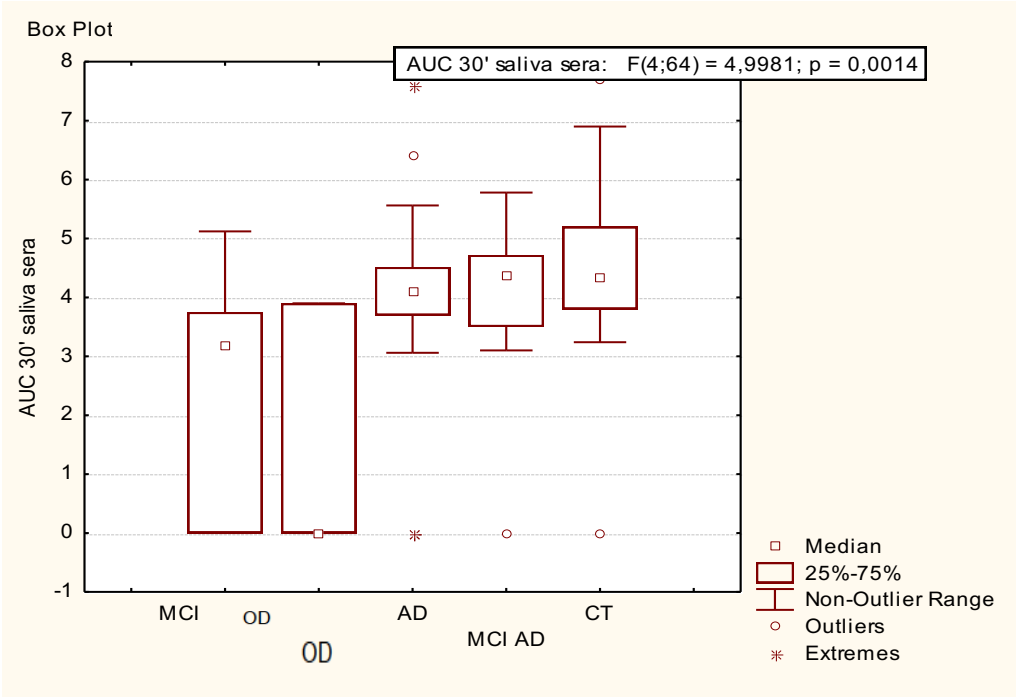

Figure 11- *Box plot showing AUC30 distribution between subgroups.*

These findings in AD and OD groups did not statistically depend on age, sex, disease severity or sleep quality.

However, in OD group the small female subgroup (3 patients) was characterized by older age, an higher disease severity and duration, an huge t-Tau amount in CSF, a worse sleep quality and a decreased melatonin secretion in biological fluids (***Table 4***). These findings could not be statistically evaluated because of the small sample size.

	Age	sd	MMS Ec	sd	Disease duration	sd	t-Tau CSF (pg/ml)	sd	PQI	sd	ESS
Male OD (n=15)	72,2	6,6	24,1	4,9	22,4	13,7	303,5	137,9	5,9	4,1	6,6
Female OD (n=3)	80,1	1,5	21,5	3,5	64,7	53,2	666,3	169,7	9,0	2,0	6,7
	sd	**DLMO**	**sd**	**AUC**	**sd**	**AUC30**	**sd**	**Melat Blood (pg/ml)**	**sd**	**MelatCSF (pg/ml)**	**sd**
Male OD (n=15)	7,6	21:00	1:34	7,7	4,8	2,3	2,1	13,4	7,0	8,0	10,2
Female OD (n=3)	4,0	nv	nv	3,4	0,0	0,0	0,0	10,0	2,6	3,9	1,3

Table 4- *Sleep, disease and melatonin secretion data in OD patients according to sex distribution.*

In 18 OD and in 5 AD patients melatonin secretion was also tested in the morning time around 8.00 a.m. in different biological fluids (saliva single spot, CSF, blood). In OD patients the morning melatonin values showed a positive correlation among the morning melatonin values in the different fluids ***(Figure 12 to 14).*** Furthermore, a positive correlation among the morning melatonin values with the evening saliva AUC values was not proven for all patients ***(Figure 15).***

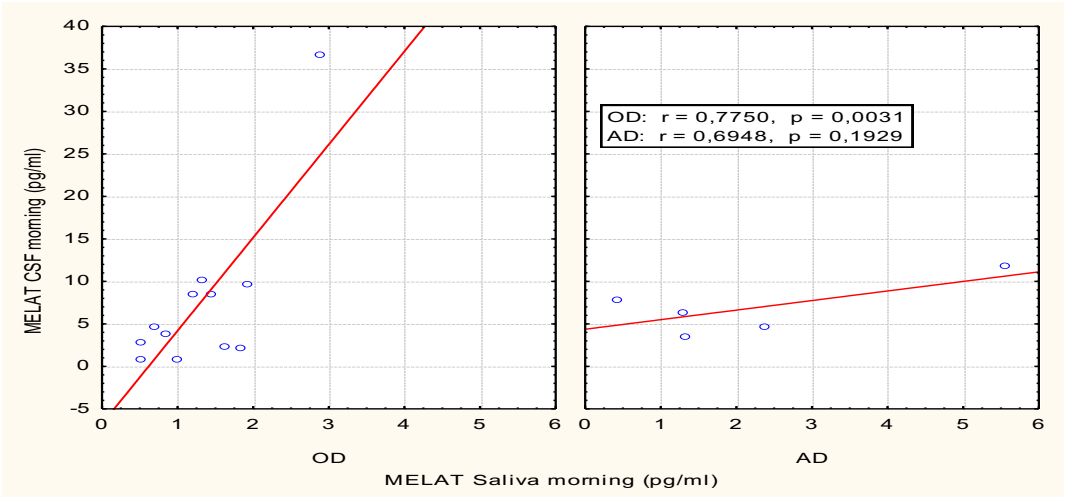

Figure 12- *Scatter plot showing morning CSF melatonin measure compared to morning saliva melatonin concentration in OD and AD patients.*

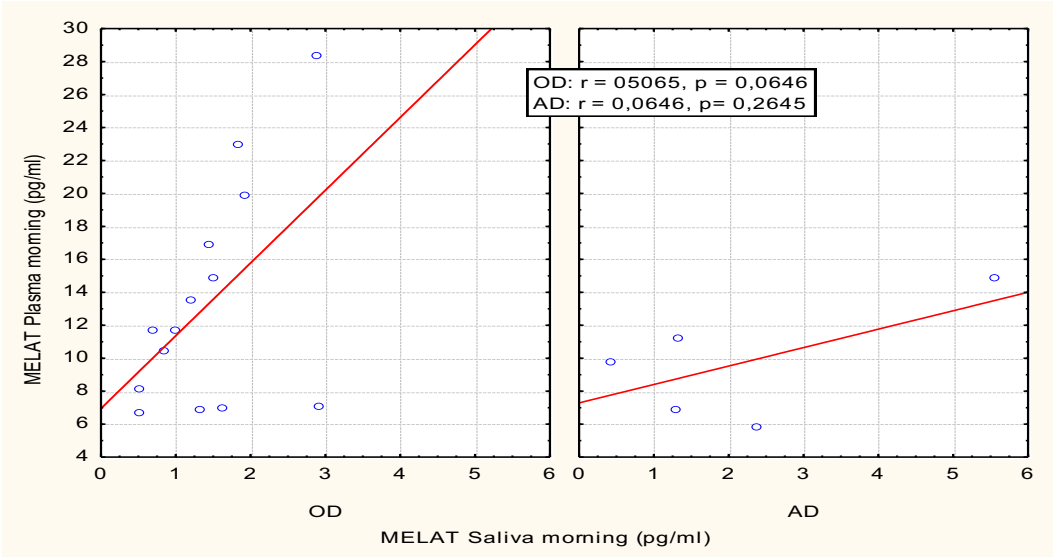

Figure 13- *Scatter plot showing morning blood melatonin measure compared to morning saliva melatonin concentration in OD and AD patients.*

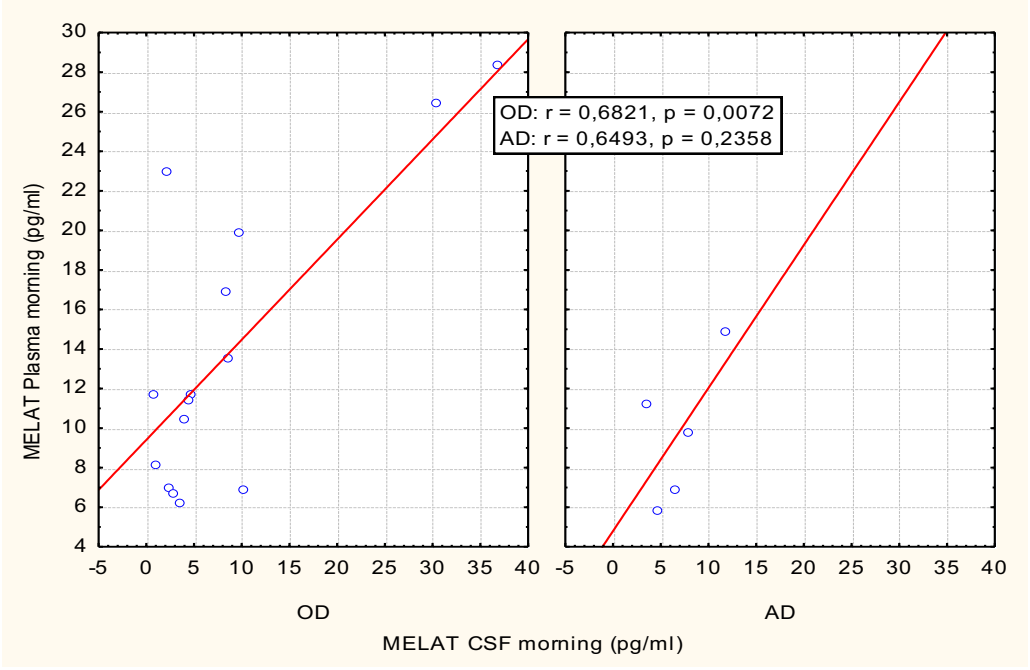

Figure 14- *Scatter plot showing morning blood melatonin measure compared to morning CSF melatonin concentration in OD and AD patients.*

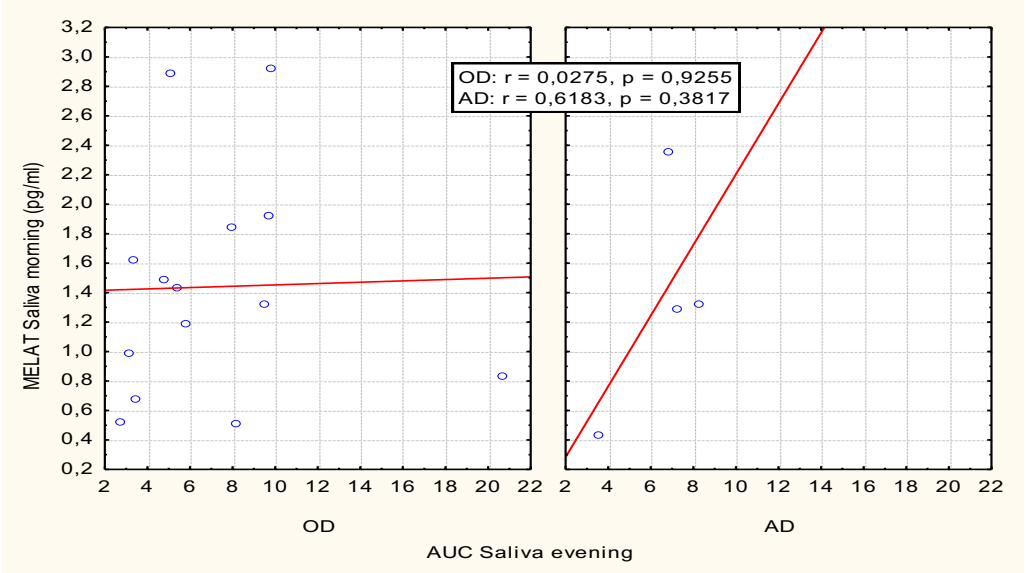

Figure 15- *Scatter plot showing morning saliva melatonin measure compared to total evening saliva melatonin concentration in OD and AD patients.*

2.2.4 Discussion

The main results of this second step were that evening melatonin secretion was confirmed to be delayed and impaired in both AD and OD groups compared to Hc, but with some important differences between groups: in AD patients melatonin secretion was more delayed and less decreased than OD patients, and this fact was not affected by factors other than disease diagnosis. Furthermore, OD and AD patients showed an higher rate of bed time at a wrong circadian phase and misperception of their circadian phase- based sleep propensity. Melatonin secretion measures in the different biological fluids proved to correlate each other at a certain timing; this finding supports the use of DLMO determination in saliva as a sensitive marker of the circadian phase in subjects with neurodegenerative diseases.

2.3 Third step: circadian phase comparison in Alzheimer's disease and other degenerative dementias at the early stage of disease

The third part of the work was focused on the use of a circadian phase modulator in order to interact with the circadian phase of AD patients and evaluate the effect on sleep and cognition. The main question we were addressing was**:** what's the clinical evidence for the efficacy of light therapy to improve sleep quality and cognition in dementias?

To answer this question at first we conducted a systematic review of scientific literature evaluating the clinical evidences about light therapy use in AD and other dementias, focusing on its effects on sleep and cognition.

After this review, taking into account these results, we performed a single blind randomised control trial to investigate the effects of a circadian-phase tailored light therapy protocol on sleep and cognition parameters in patients with Alzheimer Disease (AD) of mild /moderate severity.

2.3.1 Systematic review of scientific literature on the clinical evidence for efficacy of light therapy to improve sleep quality and cognition in dementias

2.3.1.1 Search Strategy

Pubmed

[Light therapy] AND [dementias]

AND [neurodegenerative] AND [Alzheimer]

Result: 39 papers containing direct information

relevant for the question:

6 reviews, 14 randomized controlled trials.

The Cochrane review was included.

Cochrane

Light therapy *, dementia *

Result: 1 review

2.3.1.2 Search Outcome

From the 39 papers, we considered the 33 studies having an experimental design based on the light therapy used as a stand-alone therapy. Worthy of note, 31 of the 39 initial papers and the Cochrane review were already included in the review and meta-analysis performed by R. O'Caoimh and collegues in 2019.

2.3.1.3 Results

Source	Design	Sample Size (intervention)	Intervention (duration)	Results
Satlin et al. [41]	Open trial, Within-subject	N=10 Patients with AD dementia; mean age 70.1; 90% male Mean MMSE 0.6 (SD 1.1)	Bright continue light therapy (2 h light box 2000 lux) every evening (17.00-21.00) for one week	Improved sleep-wake Cycle (actigraphy); no change in agitation
Mishima et al. [42]	Open trial with control group	N=24 (14 cases) Persons with dementia -AD or vascular; mean age 75; MMSE Moderate-severe	Bright continue light therapy morning (2 h light box 3000-5000 lux) versus dim light (four weeks)	Increased nocturnal sleep, Decreased in daytime sleep, Improved agitation (actigraphy)
Van Someren et al. [43]	Open trial, Within-subject	N=22, Persons with dementia – mixed subtypes; mean age 79 (2) ; 32% male41% male MMSE severe based on FAST scale	Bright continue light therapy – all Day (790 a 2190 lux): Increased illumination in the living rooms (four weeks)	Improved sleep-wake Cycle (actigraphy)
Colenda et al. [44]	Open trial, Within-subject	N=5, Persons with AD; mean age 76.4; Mean MMSE N/A	Bright continue light therapy (light glasses 2000 lux) every Morning (ten days)	No change in nighttime Waking (actigraphy)
Mishima et al. [45]	RCT Single blind Cross over	N=22 (12 with vascular) Residents with dementia – AD and vascular; mean age 79.6; 41% male. Mean MMSE 8.45	Bright continue light therapy - Morning (2 h light box 5000-8000 lux) versus dim light (two weeks)	Reduced nighttime Activity (rest activity monitoring)

Okumoto et al. [46]	Case study	N=1 Resident with vascular dementia; Age 89, female Mean MMSE N/A	Bright continue light therapy –(2 h light box 4000 lux) Morning (two exposures, daily for 34 and 46 days)	Increased nocturnal Sleep (sleep log)
Rheaume et al. [47]	Case series	N=3 Persons with AD dementia; mean age 70.6; 100% male, Mean MMSE N/A	Bright continue light therapy (light room; non-specified)	Increased nocturnal sleep, Reduced agitation (actigraphy)
Koyama et al. [48]	Open trial, Case series	N=6 Residents with dementia; mean age 86.2; 16.6% male, Mean MMSE N/A	Bright continue light therapy – morning or lunch (light box 4000 lux ; range 30-96 days)	Increased nocturnal sleep, (sleep log) Decreased in daytime sleep
Lyketsos et al. [49]	RCT Single blind Cross over	N=15. Residents with dementia; mean age 80.8; 6.6% male Mean MMSE 6.4 (SD 6.8)	Bright continue light therapy versus dim light (1 h daily four weeks)	Increased nocturnal Sleep (sleep log)
Yamadera et al. [50]	Open trial, Within-subject	N=27 Persons with AD dementia; mean age 79.9; 40.7% male Mean MMSE 7.8 (SD 5.2)	Bright continue light therapy – Morning (light box 3000 lux, 2 h daily h 9-11 am four weeks)	Increased nocturnal sleep, Improved sleep-wake cycle (actigraphy) Increase MMSE score
Ito et al. [51]	Open trial, Within-subject	N=14 Persons with AD; mean age of all 78.3; 43% male Mean MMSE 10.1	Bright continue light therapy – Morning (light box 3000 lux, 2 h daily h 9-11 am , 8 weeks)	No change after four Weeks (actigraphy, MMSE)
Kobayashi et al. [52]	Open trial, Within-subject	N=10, Persons with dementia; mean age 81.2 (8.8); 40% male, Mean MMSE N/A	Bright continue light therapy - lunchtime (8000 lux in light room daily for three weeks)	Improvement in sleep disturbance, Increased nocturnal sleep (sleep log)

Ancoli-Israel et al. [53], Ancoli- Israel et al. [54]	RCT Single blind	N=99 (61 cases) Residents with severe dementia; mean age 82.3 (SD 7.6); 31.5% male Mean MMSE 5.7 (SD 5.6)	Bright continue light therapy (morning or evening light box 2500 lux, 2 h daily, h 9.30-11.30 am; 17.30-19.30 pm) versus dim red with daytime sleep restriction (10 days)	Improved rest/activity rhythm, No change in nocturnal Sleep (actigraphy)
Gasio et al. [55]	RCT Single blind	N=13 (9 cases) Residents with dementia – different subtypes; mean age 85.6; 7.7% male Mean MMSE 13.92 (SD 5.37)	Continue blue-enriched light therapy (Dawn-dusk simulation, maximum 210 lux in the evening before bedtime for 45 minutes) versus dim red light (three weeks)	Reduced sleep latency, Increased sleep duration, Reduced nocturnal activity (actimetry). No change MMSE
Fetveit et al. [56], Fetveit et al. [57], Fetveit et al. [58]	Open trial Within-subject	N=11 Residents with dementia; mean age 86.1; 9.1% male Mean MMSE 11.7 (SD 8.9)	Bright continue light therapy - Morning (light box 6000–8000 lux, 2 h per day 8.00-11.00 am for 14 days)	Improved sleep efficiency, Reduced nocturnal waking, Increased morning wakefulness (actigraphy)
Skjerve et al. [59]	Open trial Within-subject	N=10 Residents with severe dementia; mean age 79.4; 70% male MMSE mean 0	Bright continue light therapy (light box 5000–8000 lux, 2 h per day morning for 4 weeks)	No change in sleepwake Cycle (actigraphy)
Ramadan et al.[60]	Open trial crossover	N=12 Persons with AD dementia; mean age 67.3, (4.1); 83% male MMSE mean range 14-15	Bright light therapy – three types: morning evening and dim (3000 lux daily, one week rotating)	Improvement in all outcomes especially with morning BLT (sleep log; MMSE)
Dowling et al. [61] Dowling et al. [62]	RCT – single blind	N=70 (53 cases, 17 controls) Residents with severe dementia; mean age 84 (10); 18.6% male (MMSE mean 7, 0-23, SD 7)	Bright continue light therapy (light box 2500 lux, 1 h per day in the morning or afternoon for ten weeks)	No improvement in sleep, (actigraphy) Improved rest/activity rhythm in those with severe impairment

Sloane et al. [63]	RCT Crossover (cluster unit)	N=66 Persons with dementia; mean age 79; 53% male MMSE mean N/A - Mild to very severe dementia	Bright continue light therapy in public areas (2500 lux, several hours per day in the morning or afternoon for two weeks)	Increased nocturnal sleep, Inconsistent effect on daytime sleep (actigraphy)
Riemersma et al.[64]	RCT Double blind	N=94 (49 cases) Residents with dementia, mixed subtypes; mean age 85; 9.6% male Mean MMSE 14.4 (SD 6.6)	Bright continue light therapy – whole day (1000 lux, light lamp in living room) daily exposure for six weeks	Modest benefit – improved sleep duration (actigraphy), Improved cognition (MMSE), Reduced depression
Nowak et al. [65]	RCT Two-factor	N=20 Residents with (severe) dementia; mean age 85.9 (SD 6.24); 0% male Mean MMSE: 1.96 (SD 2.86)	Blue-green intermittent light therapy (cap visor 45 minutes at 8000 lux in morning or evening) versus dim red light (two weeks)	Improved nocturnal Sleep (actigraphy)
Dowling et al. [66]	RCT	N=35 (18 cases) excluding those Receiving melatonin. Residents with AD dementia; mean age 85.5; 14% male Mean MMSE 9.3 (SD 7.9)	Bright continue light therapy - Morning (light box 1 h at 2500 lux) for ten weeks	No effect of BLT on night sleep, daytime wake, or rest–activity rhythm (actigraphy)
Burns et al. [67]	RCT	N=48 (22 cases) Residents with AD/other Dementia; mean age 84.5; 27.3% male Mean MMSE 5.9 (SD 5.5)	Bright continue light therapy -(light tube 10000 lux, morning 2 h from 10-12 am) for two weeks	Improved sleep, limited effect on agitation (actigraphy)
Sloane et al. [68]	RCT Crossover design	N=17 (patient-carer dyads) Persons with dementia; mean age N/A 65% aged >80 ; 35% male Mean MMSE 12.7, (SD 9.1)	Continue blue-enriched light therapy – all day (different devices , 400 Lux at the cornea); blue-white versus red-yellow (six weeks)	Improved sleep in caregivers but not people with dementia; reduced carer burden (actiwatch)

Sekiguchi et al. [69]	Case series	N=17 Residents with mixed subtypes with mildmoderate dementia; mean age 75.5 (6.2); 65% male Mean MMSE 12.8	Bright continue light therapy (Light box 5000 lux)- Morning (1h from 9.00-10-00 am) for two weeks	Improvement of sleep Disturbance (NPI), Not useful in severe dementia
Figueiro et al. [70]	Open trial Within-subject	N=14 Residents with dementia, AD and related dementia's; mean age 86.9 (4.4); 35.7% male Mean MMSE N/A Brief Interview for Mental Status score indicating moderate-severe	Continue blue-enriched light therapy (room lamps)– all day (6.00 am-6.00 pm); blue-white (300-400 lux,daysimeter) four weeks	Increased sleep quality (questionnaires), Reduced agitation and Depression (questionnaires)
Figueiro et al. [71]	Open trial Within-subject	N=35 (patient-carer dyads) . Residents with dementia, AD and related dementia's; mean age 80.8 (7.9); 74.3% male MMSE range 12-24, mild to moderate	Continue blue-enriched light therapy (room lamps)– all day (6.00 am-6.00 pm); blue-white (300-400 lux, daysimeter) four weeks	Improved sleep efficiency in patients and carers (actigraphy)
Figueiro et al. [72]	RCT Crossover design	N=46 Residents with dementia, AD and related dementia's; Mean MMSE N/A Brief Interview for Mental Status score indicating moderate-severe	Continue blue-enriched light therapy (room lamps)– all day (6.00 am-6.00 pm); blue-white (300-400 lux, daysimeter) for two 4-week periods (separated by a 4-week washout)	Increased sleep quality (questionnaires, actigraphy), Reduced agitation and Depression (questionnaires)
Ambar Akkaoui M [73]	Case study	N=1 Resident with Lewy body dementia; Age 63, male Mean MMSE N/A Brief Interview for Mental Status score indicating mild-moderate	Intermittent blue-enriched light therapy (30 minutes Light glasses Luminette 1000 lux) Morning (8:30 am, daily for 6 weeks)	Increased sleep quality (questionnaires), Reduced agitation, daytime sleepeness, nightmares and Depression (questionnaires)

Source	Design
O'Caoimh R. [74]	Review and meta-analysis
Forbes D. [75]	Review Cochrane
Hampton T.[76]	Review
Missotten P.[77]	Review
Abraha I. [78]	Review
Miloto M. [79]	Review

2.3.1.4 Comments

Light therapy has shown great promise as a nonpharmacological method to improve behavioral/cognitive and symptoms and sleep quality in dementia. Light therapy could be delivered using bright light therapy (BLT; usually 2500 lux-10000 lux) or blue-enriched light therapy (BWL; 300-1000 lux). Worthy of note, sleep-wake cycles respond differentially to the spectral power distributions of light. Human melatonin suppression has a peak sensitivity to light close to 460 nm (blue color) thus, light with relatively more energy at short wavelengths (BWL) will be relatively more effective at affecting the circadian clock. Light sources typically used in eldercare facilities (BLT) do not necessarily provide efficacious stimulation of the circadian system. Moreover, elderly patients could show a low compliance rate to high intensity of light, as in the case of BLT. It was also shown that high correlated color temperature (CCT) polychromatic light sources (bluish-white light, BWL) during daytime hours decreased depression and agitation scores in those with dementia living in long-term care facilities. The same lighting improved subjective and objective measures of sleep.

Literature shows that continue bright light therapy is the most common way to use bright light therapy in dementia (BLT, n=26 studies), although in the recent studies intermittent and continue blue-enriched light therapy has been introduced (bluish-white light, BWL, n=7 studies). Light boxes or lamps were the most used devices. The BLT and BWL studies examined different types of artificial light delivered in sessions over one-week up to 10-weeks.The duration and timing of the light varied with some studies examining light exposure over a single hour, others throughout the day; others at set periods (morning, lunchtime or afternoon). Eight studies used a dim (red) light control group as a comparison.

Outcomes were markedly heterogeneous between studies. Among the BLT

studies using actigraphy recording to evaluate sleep, the majority of them found a sleep quality improvement (12 of 19 studies, 63%). The percentage increases adding among the same studies, the ones in which a positive effect of BLT on diurnal agitation has been found (17 of 19 studies, 90%). The same results are evident taking into account the BLT and BWT randomized controlled trials (RCT, 14 studies).

Among BWL studies, most studies found a significant effect of light treatment on sleep quality measured by actigraphy recording (6 of 7 studies, 86 %).

Of those studies measuring changes in cognition (n=15), only five showed an improvement in any measures of cognition with a sleep intervention using BLT or BWT. The RCT study with the largest sample size in literature, performed by Riemersma and colleagues, suggested that six-weeks of BLT produced a sustained (at two years) improvement in ADL in dementia.

A recent Cochrane review included 8 studies that met their criteria for inclusion in their review. The authors concluded that there is not enough evidence to justify the use of light therapy to improve sleep and behaviour in persons with ADRD. However, the authors analysed studies that used a variety of light therapy approaches and, critically, it is uncertain how the actual light doses received by the study participants were measured or monitored. This is an important point to consider, because in studies where carefully controlled light stimulus was delivered, researchers in fact did find a positive impact of light on the sleep quality of persons with dementia. Moreover, outcomes measures methods about sleep and cognition were markedly heterogeneous between studies.

2.3.1.5 Clinical bottom line

Light therapy had some, albeit limited evidence supporting its use in sleep disturbances and agitation in persons with cognitive impairment, with most studies examining light exposure reporting positive effects on at least one sleep measure. However, light therapy, including BLT, did not show any significant effect in 4 recent systematic reviews and meta-analysis including a Cochrane review specifically examining the results of RCTs. This suggests that the evidence supporting light therapy is at best equivocal due to the heterogeneity in sample size, degree and subtype of dementia, study design, type of light therapy and devices used. The future studies must include randomized controlled trials on specific subtypes of dementia, using preferentially blue-enriched light therapy

carefully controlled, which effect must be verified with objective measures.

Taking into account this evidences, we performed a single blind randomised control trial to investigate the effects of a tailored light therapy protocol on sleep and cognition parameters in patients with Alzheimer Disease (AD) of mild /moderate severity.

2.3.2 Circadian phase tailored light therapy in Alzheimer disease: new findings on sleep and cognition

2.3.2.1 Rationale

This study aimed to investigate the effects of a tailored light therapy protocol on sleep and cognition parameters in patients with Alzheimer Disease (AD) of mild /moderate severity. Light was administered in accordance to circadian phase as measured by DLMO, in order to influence more properly the circadian clock.

2.3.2.2 Material and methods

2.3.2.2.1 Study Protocol

The study employed a randomized, placebo-controlled, single-blind design over a three-months period. Patients were randomly assigned to one of two conditions (treatment and sham). The randomization list was generated through a simple randomization method using "Random Number Generator" software available at www.regione.emilia-romagna.t/sin_info/generatore. The algorithm used in this site coincides with a Lehmer generator (congruential multiplicative generator). Allocation concealment is guaranteed by a central randomisation at a remote and independent location from the enrolment site.

Participants were recruited from Neuropsychology/Alzheimer's Disease Assessment Unit of IRCCS Mondino Foundation in Pavia. All the patients obtained a confirmed diagnosis of AD, based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria (McKhann et al criteria, 2011).

Considering that an advanced neurodegeneration would reduce the impact of the light therapy on regulating circadian rhythms, because of the excessive loss of mRGCs in the retina of AD patients[79], we planned to recruit only patients with mild/moderate cognitive impairment, accepting a range of MMSE score between 16 and 24.

Exclusion criteria included the presence of Obstructive sleep apnea (OSA) with an Apnea–Hypopnea Index (AHI) > 15, and the detection of Periodic limb movements (PLMs) in sleep (hourly index > 10) at a full night cardiorespiratory polygraphic recording. Patients subsequently selected to follow the light treatment protocol should not present ocular contraindications: cataract,

degenerative macular retinopathy, narrow angle glaucoma. None of the subjects enrolled were assuming psychostimulants, sedatives, antidepressant drugs or melatonin. Considering that mood disorders may influence sleep and cognition, we evaluated depressive symptoms by means of a psychometric test, the Beck Depression Inventory, administered before and after the 4-weeks light treatment. Major depression was not found in any of the participants.

Participants received the first battery of neuropsychological tests (MMSE, MOCA, NPI) at the Neuropsychology/Alzheimer's Disease Assessment Unit (IRRCS Mondino Foundation). Subjects were subsequently followed at the Unit of Sleep Medicine and Epilepsy (IRCCS Mondino Foundation) for the other steps of the experimental protocol. During the first interview patients completed sleep questionnaires (PSQI, ESS, SCI, SCADS), MEQ, and Beck depression inventory. Questionnaires were filled out under medical supervision. Participants also underwent a full night cardiorespiratory polygraphic recording, in order to rule out OSA and PLMs coexistence. The following step consisted of a 7-day/night actigraphic monitoring through a triaxial actigraphic watch (MotionWatch8) equipped with an ambient light sensor. Subjects received the actigraphy watch with a sleep diary for documenting sleep habitudes and behavior during the 7-day period. BÜHLMANN Saliva Collection Device was provided to the patients, in association with a written guidance on the method of sample collection. Participants and their caregivers were carefully instructed and also received a specific diary for documenting collection times and bedtime. ELISA was subsequently performed by the Laboratory of Functional Neurochemistry, IRCCS Mondino Foundation. The analysis involved a competitive immunoassay using a capture antibody (Ab) technique. The antimelatonin Ab was coated onto the microtiter plate. After the first 16–20 h overnight incubation, melatonin present in cases and controls as well as in the calibrators competed with biotinylated melatonin during the second 3-h incubation for the binding sites of this highly specific Ab. After washing, the enzyme label, streptavidin, conjugated to horseradish peroxidase is added, this binds during the third incubation step (1 h) to the melatonin-biotin Ab complexes captured on the coated wells. The unbound enzyme label was then removed by the second washing step, and tetramethylbenzidine substrate was added to the wells. In the fourth 30-min incubation step, a chromophore was formed in inverse proportion to the amount of melatonin present in the sample. The intensity of the color was measured at

450 nm. The concentration of melatonin was determined in picograms/milliliter (pg/ml). After DLMO determination participants returned to the sleep unit for receiving light glasses. Luminette®'s functioning is very simple and just require a minimal functional capacity. Our study is the first protocol, involving AD patients, where light therapy administration was suited on specific patient's circadian phase. Melatonin phase response curve was in fact used as a marker of circadian phase and consequently as a guidance for delivering light treatment. In order to resynchronize more appropriately the circadian rhythm[80], light was delivered for 20 minutes after spontaneous wake up in the morning in patients with a late circadian phase (LCPpts) and 1 hour before DLMO in patients with an early circadian phase (ECPpts).Once light treatment was completed subjects repeated the initial evaluations: all the participants performed neuropsychological test and sleep questionnaires. A new DLMO determination permitted to determine circadian phase shift. Seven-day/night actigraphic monitoring was repeated after Light therapy/Sham therapy in order to evaluate variations of the primary outcomes.

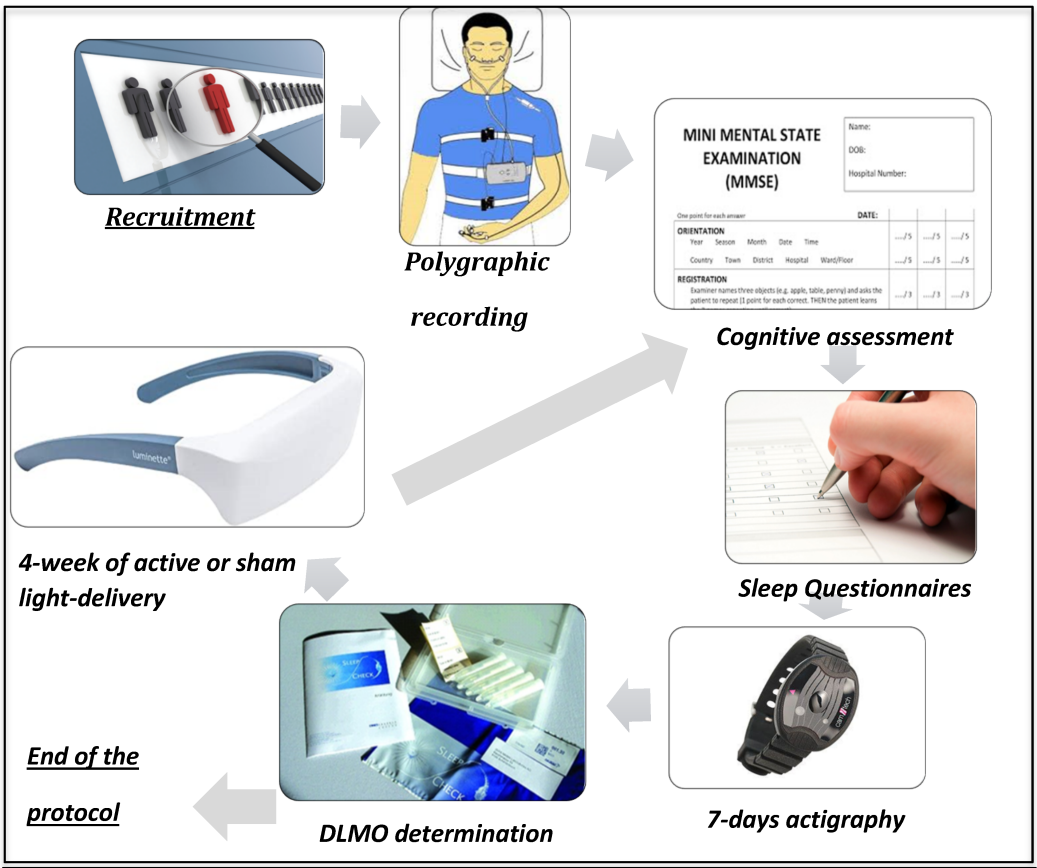

Figure 16- *Progressive steps of the study protocol.*

***Fig. 16**:* After the recruitment, all the patients once checked for clinical exclusion criteria, performing a full night cardiorespiratory polygraphic recording, and receiving the first battery of neuropsychological tests (MMSE, MOCA, NPI) with the addition of Beck Inventory. Subsequently they filled out sleep questionnaires (MEQ, SCI, SCADS, PSQI, ESS) under medical supervision. The following step consisted of a 7-day/night actigraphic monitoring through a triaxial actigraphic watch (MotionWatch8), assessing subjects' 24-hours activity-rest rhythm and objective sleep parameters. Salivary melatonin collection was then performed to determine patients' circadian phase by means of DLMO. This data permitted us to plan a 4-weeks tailored light (active or sham) exposure by means of Luminette glasses. At the end of the protocol we reassessed cognitive, sleep and circadian measures in order to evaluate a potential favourable effect of light delivery.

2.3.2.2.2 Patients

Sixteen patients were asked to participate into the study. Two patients declined the proposal, so that 14 pts could be enrolled. One out of the enrolled patients did not complete the protocol, because of the appearance of ocular irritation during the light delivery. Consequently, the present investigation definitely pertains to thirteen AD patients (male/female: 9/4; mean age 74 ± 5.4 years) *(**Table 5)**.* In two subjects of the Sham group, DLMO cannot be calculated due to curve secretion irregularities. ("bizarre curves"). One patient dropped out before performing the second actigraphic monitoring, after having completed 4-weeks light delivery and repeated DLMO determination. Another patient had to repeat salivary melatonin collection because the quantity of saliva in cotton swabs was insufficient.

Attribute	Quantity
Participants (number)	**13 (9 M/ 4 F)**
Age (years; SD)	**74 ± 5.4**

***Table 5-** Demographic and clinical features of patients*

MMSEc score, mean (SD)	**21 ± 4**
MOCAc score, mean (SD)	**16 ± 4**
Luminette protocol (number, %)	**8 (61,5 %)**
Sham protocol (number, %)	**5 (38,5%)**

Table 6- *MMSEc = Mini Mental State Examination; MOCA = Montreal Cognitive Assessment*

2.3.2.2.3 Outcome measures

2.3.2.2.3.1 Subjective sleep parameters

Four questionnaires were administered before and after the 4-weeks light therapy to assess subjective sleep quality. Pittsburgh sleep quality index (PSQI) and Epworth sleepiness scale (ESS) represented secondary outcomes of our study. The PSQI is a tool to measure sleep quality in clinical populations. It is composed of 19 items that generate 7 component scores (sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction). The sum of the 7 component scores yields one global score. Global scores > 5 points indicate sleep disturbances.

The ESS is an eight-item assessment of somnolence, with possible scores from 0 to 24. A score of over 10 is considered indicative of an abnormal daytime sleepiness.

Sleep continuity scale in Alzheimer's disease (SCADS) and Sleep condition indicator (SCI) were included reinforcing PSQI evaluation.

2.3.2.2.3.2 Neuropsychological assessment

Neuropsychologists from Neuropsychology/Alzheimer's Disease Assessment Unit (IRRCS Mondino Foundation) administered neuropsychological tests at the beginning of the protocol and by a week after light delivery. MMSE and Montreal Cognitive Assessment (MoCA) are widely used screening assessment for detecting cognitive impairment. The study provided MMSE score corrected

according to age and education as a secondary outcome of the study. In addition, patients received the Neuropsychiatric Inventory (NPI) in order to assess the most typical behavioural disturbances occurring in dementia patients (delusions, hallucinations, dysphoria, anxiety, agitation/aggression, euphoria, disinhibition, irritability/lability, apathy, and aberrant motor activity).

2.3.2.2.3.3 Objective sleep parameters

A 7-day/night actigraphic monitoring was performed, before and after light treatment, by means of a triaxial actigraphy watch (MotionWatch 8) worn at the sleep unit and held at home. This anobstrusive wristworn device is validated for recording daytime/nightime physical activity and light exposure, by means of an accelerometer and an in-built ambient light sensor with event marker. The actigraphic recording permitted to assess the following objective sleep parameters: Sleep efficiency (SE), Total sleep time (TST), Wake after sleep onset (WASO), Fragmentation Index, Midsleep time, Interdaily stability (IS), Intradaily variability (IV), Bedtime (BT). Sleep efficiency is the actual sleep time expressed as a percentage of time in bed. Total sleep time is the amount of actually sleep time in 24-hours sleep episodes; this time is equal to the total sleep episode less the awake time. Seven-day mean values of SE and TST were considered as the first outcome of the study, because of their better reliability in detecting variations of sleep parameters compared to the questionnaires. Interdaily stability and Intradaily variability are deemed to be measures of the strength of circadian rhythmicity. Interday stability reflects the degree of consistency of activity patterns from one day to the next; values range from 0 to 1, with higher values indicating greater stability. Intradaily variability reflects the fragmentation of the rest-activity rhythm, that is, the rate of shifting between rest and activity. High intradaily variability may indicate daytime napping and/or nighttime arousals; values range from 0 to 2, with higher values indicating greater fragmentation.

2.3.2.2.3.4 Circadian rhythm assessment

In our protocol study circadian rhythm was assessed by means of three parameters. Morningness-Eveningness Questionnaire (MEQ), a multiple-choice questions test which represents a subjective circadian phase marker; objective measures of IS and IV, obtained by actigraphy; parameters calculated from

endogenous melatonin secretion semicurve (DLMO, AUC), further objective indexes of circadian rhythm.

The Dim Light Melatonin Onset (DLMO) as the moment the serotine melatonin production during dim light conditions cross a threshold value (10 pg in the plasma and 3 pg in saliva). DLMO is deemed a reliable marker of the circadian phase. Accordingly to the literature [26], categorical DLMO permitted us to consider circadian phase as “early” (DLMO before 19:30), “intermediate” (DLMO between 19:30 and 22:00), and “late” (DLMO after 22:00).

The estimation of salivary melatonin secretion was provided by means of BUHLMANN saliva melatonin ELISA kit(EK-DSM). This quantitative and highly sensitive test is based upon the Kennaway G280 antibody which uses a sodium hydroxide pretreatment of the sample followed by neutralisation with hydrochloric acid.

Participants were instructed to collect saliva samples at five time points, during the four hours before usual bedtime, and one hour after, setting an alarm. Subjects were carefully instructed and received written guidance on the method of sample collection. Saliva was collected using inert polymer cylindrical swabs (10 mm x 30 mm), which were then placed in a storage tube with a snap cap and kept in the refrigerator until delivery to the lab. Cotton swab provided in the device was kept in the patient's mouth below the tongue for about 3 minutes. The participants documented collection times by means of a specific diary, consigned in association with the melatonin kit in the Unit of Sleep Medicine and Epilepsy (IRCCS Mondino Foundation). Upon receipt, tubes were centrifuged and stored at -20°C until the analyses. ELISA was performed at the Laboratory of Functional Neurochemistry, IRCCS Mondino Foundation.

The assay permitted the calculation of the following parameters: DLMO, AUC (area under melatonin secretion curve) and AUC30 (first 30’ after DLMO). The AUC quantifies the amount of melatonin secretion during the test period. Phase angle between objective bedtime (by actigraphic monitoring) and DLMO were subsequently calculated.

Patients’ chronotype resulted by salivary melatonin test was compared to MEQ scores, in order to identify the presence of an accordance/discordance between self-reported and objective chronotype.

2.3.2.2.3.5 Light treatment parameters

Considering that AD patients when compared to age matched control have a greater loss of mRGCs, and these cells are particularly sensitive to monochromatic blue light (at 470 nm), we used this selective wavelength for increasing the effectiveness of tailored light therapy on circadian dysfunction. The light treatment was administered using specific glasses (Luminette®). This medical device, produced by the company Lucimed called Luminette® (EAN: 0702382929671), weighs 0.6 kgs and has the following dimensions: 22×11×11 cm. The device provides daily exposure to a blue-enriched light of 10,000 Lux perceived, possessing a UV filter and CE standards. Luminettes® of the study were set on intensity 3/3, and worn 20 minutes per day, during a consecutive period of 28 days. The glasses allow control of the light exposure and good ergonomics, with the possibility for the patient to continue its normal activities. This device is freely available on the market, and the technical details of the Luminette® are available on demand.

A control exposure (Sham treatment) was also performed by the patients, using the same Luminette® device and CE standards, but offering a fluorescence of 50 Lux, close to ambient light, and therefore considered ineffective.

Light delivery was synchronized to the peculiar circadian rhythm of the participants, accordingly to DLMO estimation. Consequently patients with a late circadian phase (LCPpts) wore Luminette® after spontaneous wake up in the morning, while subjects presenting an early circadian phase (ECPpts) performed the light therapy 1 hour before DLMO. Subjects with a DLMO between 19:30 and 22:00 was considered possessing an intermediate circadian phase, while patients whom DLMO was out of this time range was associated to an extreme chronotype.

2.3.2.3 Results

2.3.2.3.1 Circadian analyses

Light therapy induced a circadian phase shift in 7 out 8 patients exposed to Luminette (range 21-166 minutes) ***(Figure 17).***

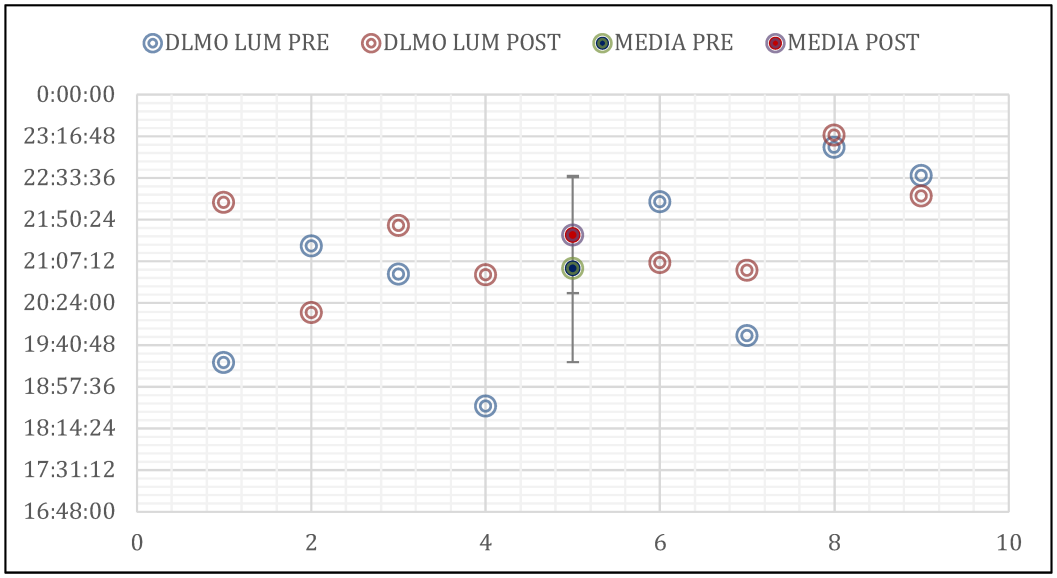

Figure 17- *DLMO shift before (PRE) and after (POST) 4-weeks Luminette protocol.*

DLMO = Dim Light Melatonin Onset; LUM = Luminette protocol. After a single-blind 4-weeks tailored light therapy, AD patients presented a circadian phase shift towards a later chronotype. This data is probably the result of a more consistent circadian phase shift in the ECPpts compared to LCPpts.

About Luminette exposure, 6 out 8 patients presented a phase shift in accordance to the melatonin phase response curve (PRC), with a mean shift time of 84.14 ± 49.4 minutes ***(figure 18).*** In the five patients treated with sham, DLMO cannot be calculated due to curve secretion irregularities, it was unchanged in one patients while it was shifted in the other two ***(figure 18).***

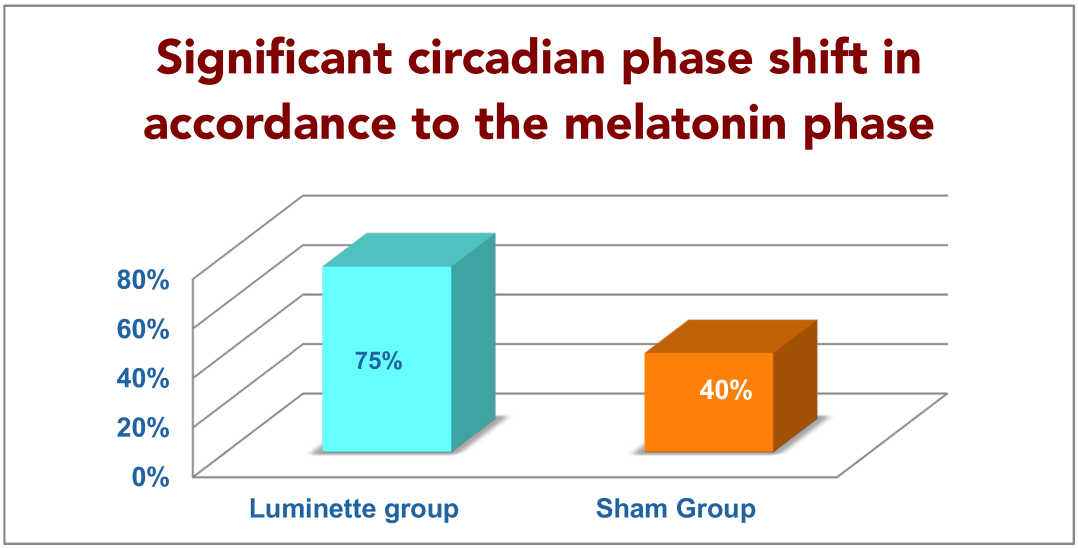

Figure 18: *Significant circadian phase shift in accordance to the melatonin phase: percentage of responders in both groups.*

In the group of 8 AD patients treated with Luminette the circadian phase shift was wider, but not significantly, in the ECPpts subgroup (91 ± 63 minutes, p=0.063) than in LCPpts subgroup (32.3 ± 26.8 minutes, p not significant) ***(Table 7).***

In Luminette group as a whole the DLMO global AUC was not significant modified after light therapy (AUC at the baseline was 3.8 ± 0.7; after the light therapy it was 3.5 ± 0.6, p not significant) regardless of early or late circadian phase. In fact the ECPpts AUC at the baseline was 3.5 ± 0.01, after the light therapy it was 3.4 ± 0.5, p not significant. In the LCPpts the AUC at the baseline was 4.4 ± 0.9; after the light therapy it was 3.9 ± 0.8, p not significant. Considering the DLMO AUC 30 we found a reduction after light therapy (AUC30 at the baseline was 1.3 ± 0.6; after the light therapy it was 0.7 ± 0.6, p=0.01), without any significant difference between ECPpts and LCPpts (In ECPpts AUC30 at the baseline was 1.1 ± 0.3; after the light therapy it was 0.5 ± 0.3, p not significant; in LCPpts AUC30 at the baseline was 1.7 ± 0.9; after the light therapy it was 1.2 ± 0.7, p not significant).

TAB 2	AD		LUMINETTE		SHAM		ECPpts (LUM)		LCPpts (LUM)	
	PRE	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	POST
N	13	13	8	8	5	5	2	2	3	3
DLMO	20:53:55 (± 1:53:18)	21:49:23 (± 1:15:58)	20:59:30 (± 1:36:00)	21:34:00 (± 0:58:07)	20:46:00 (± 2:22:00)	22:13:00 (± 1:40:00)	18:59:30 (± 00:31:00)	21:30:00 (± 00:53:00)	22:36:40 (± 00:28:00)	22:13:00 (± 1:06:30)
AUC30'	1.21 (± 0.68)	0.61 (± 0.53)	1.32* (± 0.57)	0.68* (± 0.60)	1.03 (± 0.88)	0.48 (± 0.44)	1.09 (± 0.30)	0.54 (± 0.30)	1.68 (± 0.92)	1.17 (± 0.76)
AUC	3.78 (± 0.69)	3.37 (± 0.47)	3.76 (± 0.70)	3.46 (± 0.57)	3.79 (± 0.69)	3.21 (± 0.20)	3.52 (± 0.17)	3.36 (± 0.45)	4.36 (± 0.95)	4.88 (± 0.85)
Midsleep	03:30:00 (± 1:06:38)	02:53:00 (± 0:59:35)	03:20:00 (± 00:54:00)	03:12:00 (± 00:52:10)	03:43:00 (± 1:08:00)	02:23:00 (± 1:30:00)	02:24:00 (± 00:29:45)	02:53:30 (± 00:14:00)	04:21:00 (± 0:18:34)	03:58:00 (± 0:15:50)
MEQ	58.31 (± 6.18)	62.54 (± 5.42)	57.25 (± 6.00)	62.25 (± 6.15)	59.81 (± 7.20)	61.17 (± 6.84)	60.00 (± 14.00)	68.89 (± 26.90)	55.45 (± 2.67)	58.32 (± 4.60)
IS	0.62 (± 0.14)	0.60 (± 0.23)	0.63 (± 0.07)	0.64 (± 0.09)	0.65 (± 0.13)	0.51 (± 0.28)	0.65 (± 0.02)	0.61 (± 0.07)	0.63 (± 0.11)	0.62 (± 0.13)
IV	1.34 (± 0.21)	0.85 (± 0.34)	1.44 (± 0.26)	0.93 (± 0.34)	1.12 (± 0.52)	0.71 (± 0.44)	1.34 (± 0.45)	1.22 (± 0.45)	1.50 (± 0.07)	0.93 (± 0.06)

Table 7: *Circadian parameters before (PRE) and after (POST) Luminette/Sham protocol.*

ECPpts = Early Circadian Phase patients; LCPpts = Late Circadian Phase patients; DLMO = Dim Light Melatonin Onset; AUC30' = Area Under the Curve thirty minutes after DLMO; AUC = Area Under the Curve (salivary melatonin secretion curve); MEQ = Morningness Eveningness Questionnaire; IS = Interdaily Stability (0-1); IV = Intradaily Variability (0-2). The presence of "" indicates a statistical significant result (p<0.05).*

Considering the global AD group, patients perceived themselves having a morning oriented chronotype (MEQ mean score 58 ± 6, percentage of subjective serotine type at MEQ score 0), while salivary melatonin dosage showed a mean DLMO at the baseline of 21:18 ± 1:34, indicating an intermediate circadian phase, with a percentage of DLMO values indicating a serotine profile in about 46 % of the subjects ***(figure 19 and 20).***

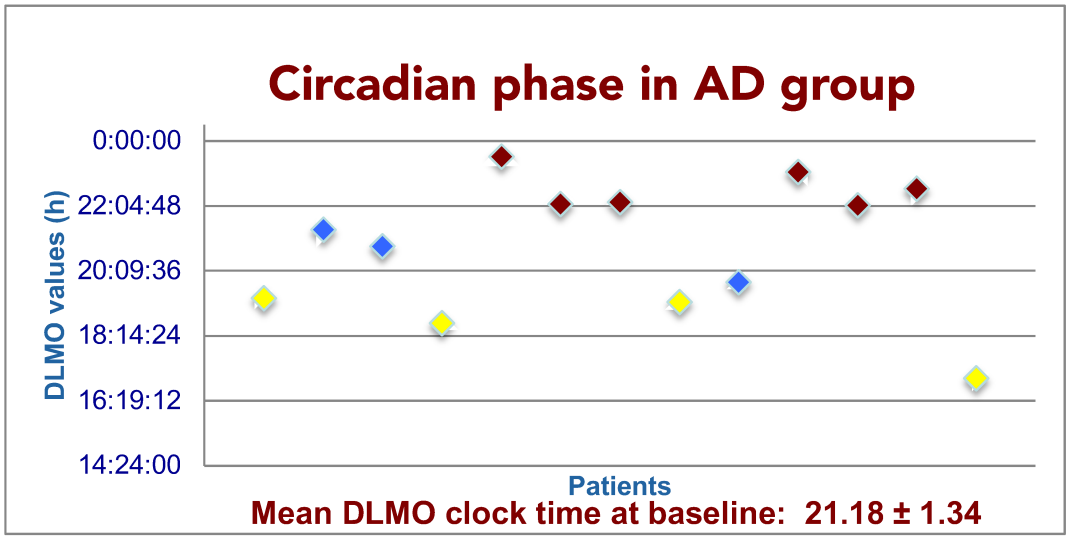

Figure 19- *Circadian phase in AD group: baseline DLMO values distribution.*

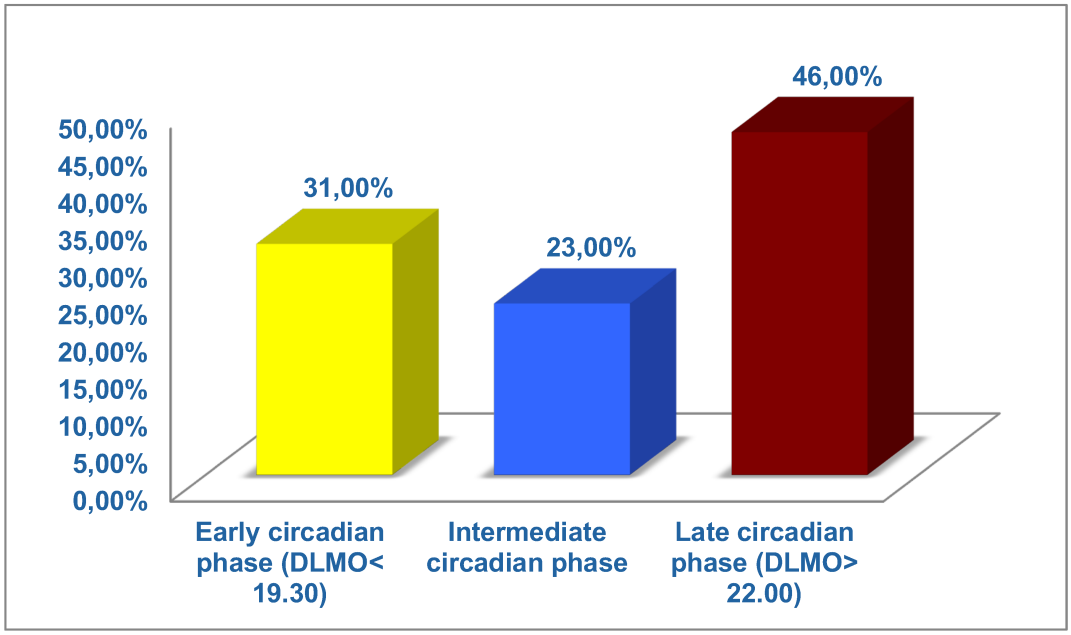

Figure 20- *Baseline DLMO values distribution according to circadian phase categories (percentage of patients).*

Furthermore, four subjects presented a midsleep between 4:00 and 5:00 am, suggesting a serotine type. Midsleep obtained by actigraphic monitoring is an objective parameter, whom association to DLMO highlights the discrepancy between subjective and objective chronotype evaluations ***(Figure 21).***

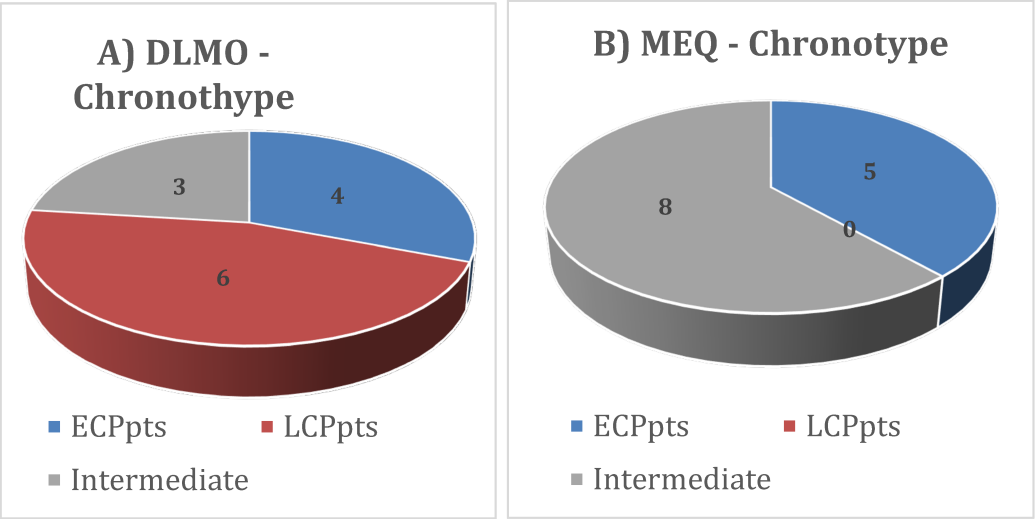

Figure 21- *Discrepancy between subjective and objective chronotype in AD patients*

Fig. 21: ECPpts = Early Circadian Phase patients; LCPpts = Late Circadian Phase patients; DLMO = Dim Light Melatonin Onset. AD patients perceived themselves having a morning oriented chronotype, with a mean MEQ score of 58 ± 6 **(B)**. Differently, mean DLMO at the baseline **(A)** indicates the presence of a late/intermediate circadian phase in all the enrolled subjects.

2.3.2.3.2 Primary outcomes

Sleep efficiency did not change both in the Luminette group and in the Sham group after the 4-week protocol (pre Luminette mean SE: 82% ± 5.16; post Luminette mean SE: 79.4% ± 4.7, p not significant; pre Sham mean SE: 82.1% ± 10.6; post Sham mean SE: 80.3% ± 15.4, p not significant). The same result is given considering ECPpts compared to LCPpts in both groups.

In Luminette group the mean 24-hour total sleep time (TST) increased after active light treatment with respect to pre treatment condition showing a positive trend which was instead absent in the Sham group (Luminette TST pre 512±66 minutes, Luminette TST post 584±99.9 minutes, p=0.27; pre Sham TST: 453 ± 124 minutes; post Sham TST: 460 ± 107 minutes, p=0.43) ***(Figure 22).*** Total sleep time was not significant modified in ECPpts (pre Luminette TST: 550 ± 57 minutes; post Luminette TST: 607 ± 180 minutes, p not significant) compared to LCPpts (pre Luminette TST: 479 ± 56 minutes; post Luminette TST: 486 ± 89 minutes, p not significant).

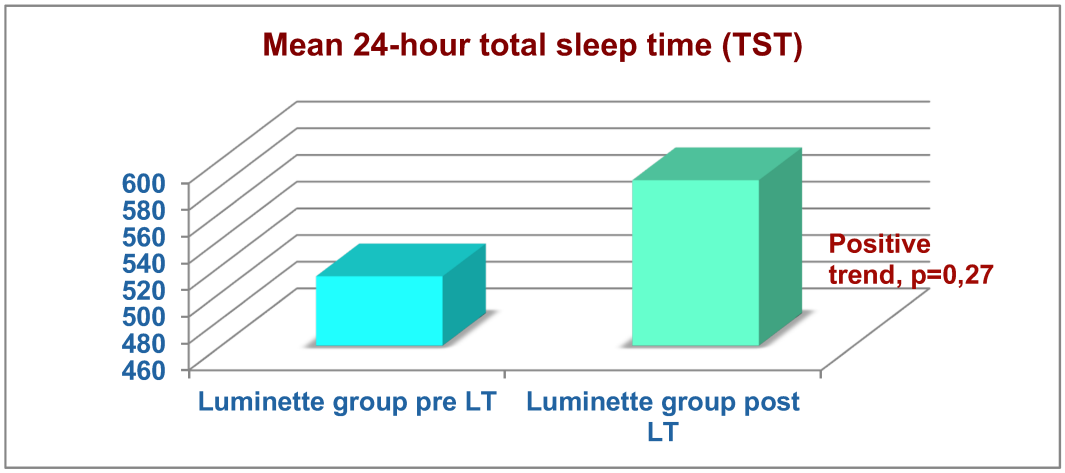

Figure 22- *Mean 24-hour total sleep variations before (PRE) and after (POST) Luminette protocol (minutes).*

In Luminette group the interdaily stability was unchanged before and after light therapy. At the baseline ID was 0.6 ± 0.07; after the light therapy it was 0.6 ± 0.09. The same result is given considering ECPpts compared to LCPpts in both groups.

Concerning the intradaily variability in Luminette group there was no substantial variation with light therapy protocol. Baseline IV was 0.8 ± 0.2; after the light therapy it was 0.9 ± 0.3.

2.3.2.3.3 Secondary outcomes

Subjective sleep quality significantly improved in Luminette group (Pittsburgh pre Luminette: 5 ± 2; Pittsburgh post Luminette: 3 ± 2, p=0.05), but remained unvaried in Sham group (Pittsburgh pre Sham: 4 ± 1; Pittsburgh post Sham: 4 ± 2, p not significant). Data are displayed in ***figure 23.*** A not significant improvement was assessed in ECPpts (Pittsburgh pre Luminette: 7 ± 1; Pittsburgh post Luminette: 2 ± 0, p not significant) compared to LCPpts (Pittsburgh pre Luminette: 5 ± 2; Pittsburgh post Luminette: 4 ± 2, p not significant).

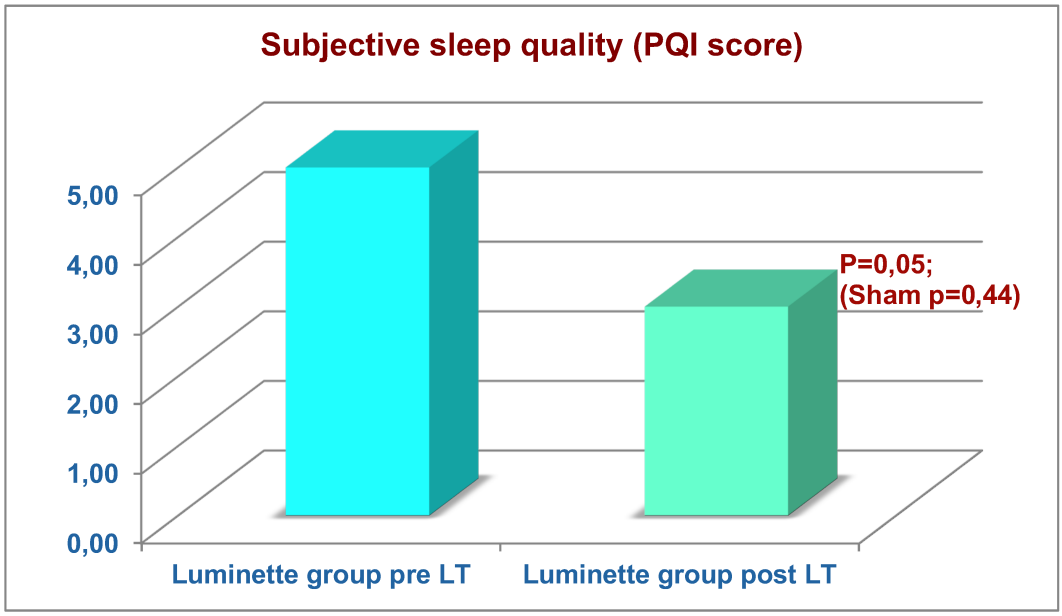

Figure 23- *Pittsburgh quality index mean value variations before (PRE) and after (POST) Luminette protocol.*

Sleepiness evaluated by means of ESS did not show a variation before and after light therapy in Luminette group (pre Luminette ESS: 4.38 ± 1.64; post Luminette ESS: 4.75 ± 1.51, p>0.05).

Sleep Condition Indicator and SCADS did not improve after light delivery in both Luminette and Sham groups.

Despite a not significant difference between pre and post light therapy MMSEc mean values (mean MMSEc pre Luminette: 19.3 ± 3; MMSEc post Luminette: 21.2 ± 1.9, p=0.07), however individual MMSEc scores improved in 6 out of 8 AD patients with active light treatment, with only 2 patients having a stable MMSEc. Differently MMSE scores worsened in three out of five AD patients on SHAM treatment ***(figure 24).***

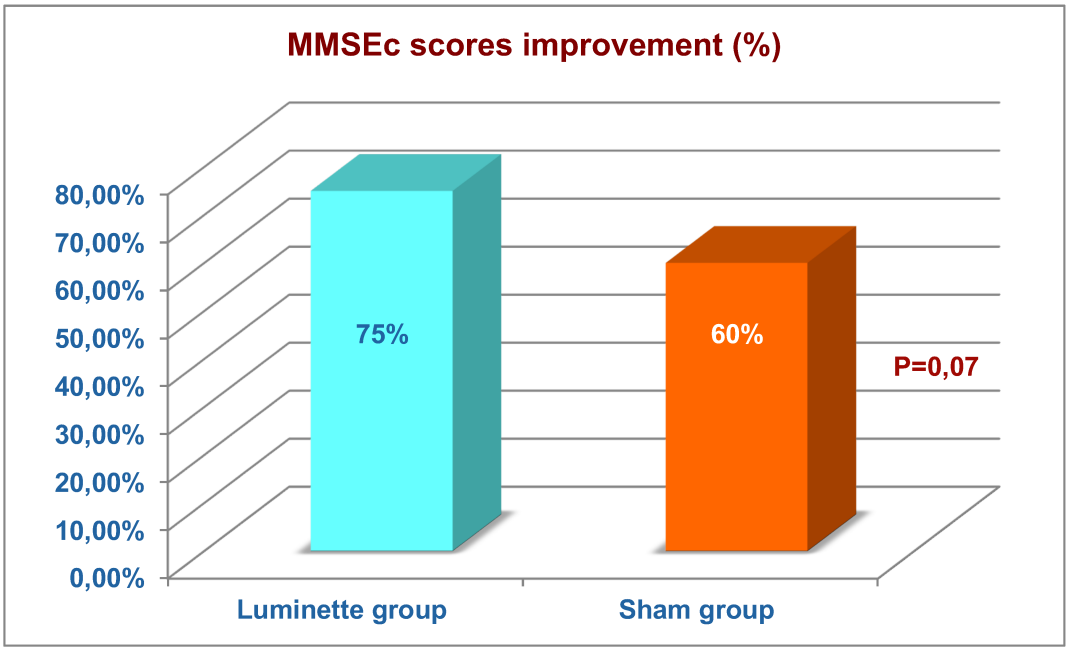

Figure 24- *Percentage of patients with MMSEc score improvement after Luminette/sham protocol.*

In Luminette group we found a greater, but not significantly, improvement in ECPpts (MMSEc pre Luminette: 18.5 ± 5.9; MMSEc post Luminette: 22.5 ± 0.7, p not significant) compared to LCPpts (MMSEc pre Luminette: 20 ± 2; MMSEc post Luminette: 22.3 ± 1, p not significant). These data were confirmed analyzing MoCa values ***(Table 25).***

Beck's Depression Inventory and NPI did not change before and after light therapy in Luminette group.

TAB 4	AD		LUMINETTE		SHAM		ECPpts (LUM)		LCPpts(LUM)	
	PRE	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*	*PRE*	*POST*
N	13	13	8	8	5	5	2	2	3	3
PSQI	4.5 (± 1.8)	3.5 (± 2.0)	5.4* (± 2.0)	3.1* (± 1.7)	3.3 (± 0.8)	4 (± 2.9)	7.0 (± 1.4)	2.0 (± 0.53)	5.3 (± 1.5)	4.3 (± 2.1)
ESS	4.0 (± 3.0)	4.2 (± 3.4)	4.4 (± 4.6)	4.7 (± 4.3)	3.3 (± 1.5)	3.1 (± 2.5)	7.0 (± 10.0)	2.5 (± 3.0)	3.7 (± 2.3)	2.7 (± 1.0)
MMSEc	21.1 (± 4.2)	22.2 (± 2.4)	19.1* (± 2.2)	21.2* (± 2.1)	23.7 (± 4.4)	23.5 (± 2.5)	18.0 (± 6.0)	22.6 (± 0.7)	20.0 (± 2.0)	22.3 (± 1.1)
MoCAc	16.0 (± 4.5)	17.0 (± 4.1)	14.5 (± 4.5)	16.7 (± 4.6)	17.8 (± 4.4)	16.8 (± 3.3)	9.0 (± 2.3)	13.0 (± 2.1)	17.3 (± 3.1)	20.0 (± 1.5)

Table 25: *Secondary outcomes: results of sleep and cognition questionnaires.*

ECPpts = Early Circadian Phase patients; LCPpts = Late Circadian Phase patients; PSQI = Pittsburgh Sleep Quality Index; ESS = Epworth Sleepiness Scale; MMSE = Mini Mental State Examination; MoCA = Montreal Cognitive Assessment. The presence of "*" indicates a statistical significant result ($p<0.07$).

2.3.2.4 Discussion

The efficacy of light therapy in improving sleep, behaviour and cognition in patients with dementia, namely AD dementia, is a matter under debate and deemed deserving further investigations. In fact the data in the literature are mixed and equivocal due to the heterogeneity in sample size, degree of dementia, study design, type of light therapy and devices of light delivery used. In order to maximize the potential beneficial effects of light therapy we planned to use enriched blue light, which the mRGCs are particularly sensitive to, and we tested AD patients at an early/moderate stage of the disease, when the status of retino-geniculate trait and nucleus suprachiasmaticus is deemed to be still relatively preserved. Furthermore we chose to tailor light exposure according to the individual circadian phase as measured by a reliable and strong circadian phase biomarker, such as DLMO. In fact in the great majority of published works, patients were exposed to bright light during the day, regardless their individual circadian phase. This could limits the efficacy of light in enhancing sleep quality, and even may aggravate the circadian misalignment in some subjects. In fact, in a previous work[81] we found that, in spite of a trend to a decreased and delayed evening melatonin secretion compared to healthy controls, AD patients present a quite remarkable degree of variability in DLMO individual values.

In this randomized placebo-controlled study, we documented that 4-weeks tailored light therapy proved to be associated to an objective phase shift in accordance to the melatonin phase response curve, a trend to an increased subjective sleep quality, 24-hour TST and cognitive performance.

Concerning our primary outcomes, there was not a meaningful effect of light on nocturnal sleep efficiency. Although 24-hour total sleep time after Luminette's protocol showed a not significant improvement ($p=0.27$), we found a positive trend to an increase, which is also suggested by others works in literature[49-42]. Interdaily stability and intradaily variability remained substantially unvaried. These findings are at odds with the results reported by Van Someren et al, Fetveit et al,

Ancoli-Israel et al, who found a major improvement of actigraphic parameters, included SE, IS and IV after light delivery[43-53].

Light also had a beneficial effect on subjective sleep quality, with a Pittsburgh Sleep Quality Index nearly significantly reduced in Luminette's group (p=0.05). Our result is in keeping with the data of Figueiro et al, Sloane et al, indicating that light exposure in AD is associated to an improvement in PSQI scores. These authors also found an effect of light on depression and agitation behavior[72-63]. In our study, Beck scale was unvaried, but there were not enrolled patients with major depression. Agitation behaviours were not reported by patients and caregivers, probably due to the early stage of the disease in our subjects.

Considering the limited sample size of the patients studied in the protocol, our results concerning sleep parameters might be influenced by one patient, who presented a robust increase in rest-activity rhytmhs consolidation, with TST increasing from 590 minutes to 734 minutes, SE being stable, and daily sleepiness and PSQI strongly improving (ESS varied from 14 to 1; PSQI from 8 to 2). This patient had also an impressive increase of MMSEc score, gaining eight points (from 14 to 22) after 4 weeks of tailored light exposure. Such a great improvement is remarkable in a neurodegenerative form of dementia, but has to be considered cautiously because of the well-known fluctuations of MMSE score.

In AD patients with active-light-exposure, cognitive performance improved after light therapy in 6 out 8 patients, while it got worse in Sham group, as we expected considering the natural progression of dementia in AD. The improvement we observed is probably linked to the fact we studied AD patients in an early stage of the disease. In fact Yamadera et al., reported a beneficial effect of bright light on MMSE scores more remarkable in early than in severe forms of AD[82], reasonably attributing this data to the fact that retino-geniculate trait and nucleus suprachiasmaticus are less damaged in early than in advanced forms of AD.

Concerning circadian phase assessment, we found a significant discrepancy between subjective and objective chronotype evaluations in AD patients. Patients perceived themselves having a morning oriented chronotype (MEQ mean score 58 ± 6), while salivary melatonin dosage showed a mean DLMO indicating an intermediate circadian phase or a serotine profile. This finding is consistent with our previous study[81], where we assessed a trend to a delay of the circadian phase in AD patients compared to controls, and a too early bedtime in respect to

their DLMO clock time. A possible explanation might be that subjective perception in AD patients is by habits distorted by tendency to self-isolation and/or misjudgment, anosognosia [37].

A favorable aspect of our protocol is its good feasibility. There was minimal risk of harm to the participants, as no known safety risks are associated with the device used in the study, except for modest and transient eye disorders[83] (conjunctival reddening, eye irritation, mild headache, eye fatigue) for which the patients were screened before and through the study. Most of the patients were able to perform the melatonin test and to complete it correctly, Luminette glasses proved to be suitable and easy to use with only one patient dropping out because of mild side effects due to light exposure (ocular irritation and burning). Enrolling patients with mild to moderate AD, we had the possibility to administer questionnaires directly to the participants, avoiding the intermediation of caregivers. Proxy data, in fact, could not provide an evaluation of subjective sleep quality perception.

3. CONCLUSION

Initial evening secretion of melatonin proves to be delayed and mildly impaired in patients with mild/moderate form of Alzheimer disease while patient's subjective sleep parameters and chronotype are reported similar to those of HC. This data indicate that, subclinical altered patterns of melatonin secretion occur in subjects with AD at an early stage of the disease independently of sleep complaints.

Comparing AD circadian phase to another type of neurodegenerative dementia as FTLD, evening melatonin secretion was confirmed to be delayed and impaired in both groups but with some important differences. In AD patients melatonin secretion was more delayed and less decreased than FTLD patients, at this fact was not affected by other factors than disease diagnosis. Furthermore, FTLD and AD patients showed an higher rate of phase angle alterations and circadian phase mispercetions than Hc. Melatonin secretion proved to correlate in the different biological fluids at a certain time among patients, an this finding support the use of DLMO determination in saliva as a sensitive marker of circadian phase. Finally, a light therapy protocol tailored on the circadian phase proved to be feasible in patients with mild/moderate forms of AD and associated to an objective phase shift in accordance to the melatonin phase response curve, a trend to an increased subjective sleep quality, 24-hour TST and cognitive performance.

4. LIMITATIONS OF THE STUDY

In step one of our studies, some limitations may be addressed. A 7-day actigraphic monitoring would have provided more reliable evaluation of sleep-wake patterns in patients and controls. Even though instrumental monitoring is not required during in-home melatonin salivary test, actigraphic monitoring along with light sensor would have provided reliable measures of rest activity patterns of the patients and of their compliance with respect to stay in a dark environment while performing the test.

In the second step of this thesis, circadian phase of FTLD patients and of 5 AD ones was investigated in hospital environment. This setting could modify melatonin secretion dynamics and homeostatic sleep pressure as well. However, a sleep medicine expert supervised every step of the circadian phase determination. Patients were also provided with sunglasses and ear plugs to protect themselves from environmental interferences during the test.

In step three results might be influenced by the predominance of males in the sample (M/F: 9/4). In fact sex related different impact on light exposure efficacy is reportedly in AD[84]; specifically there is a major improvement on sleep quality and mood in female patients. Future studies of new therapeutics for sleep disorders in AD should take into account intentional stratification by sex/gender. In our protocol there was not an assessment of lighting conditions in domestic environment. This fact represents an important limitation of the study, because we could not evaluate the “light diet” of our subjects during the 4 weeks protocol. Differently, Figueiro et al (2019) focused on subjects’ environment, by arranging appropriate lighting conditions, tailored to maximally entrain the circadian system[72]. The development of a “light diary” would be a helpful tool to assess and/or modify daily/nightly light exposure of participants in future studies. In conclusion, this randomized clinical trial, while promising for application, should be replicated using a larger sample size and perhaps using longer treatment duration (eg, 6 months).

5. PUBLICATIONS

1. **"Serotine melatonin timing secretion in real life conditions in patients with Alzheimer disease of mild to moderate severity."** Raffaele Manni, Riccardo Cremascoli,, Carlo Perretti, Roberto De Icco, Marta Picascia, Cristina Ghezzi, Silvia Cerri, Elena Sinforiani, Michele Terzaghi. *Sleep Medicine 2019.*

2. **"Systematic review of scientific literature on the clinical evidence for efficacy of light therapy to improve sleep quality and cognition in dementias."** *CAT essay. EAN examination 2020.*

3. **"Circadian phase tailored light therapy in Alzheimer disease: new findings on sleep and cognition."** *25th Congress of the European Sleep Research Society (ESRS). Supplement to the Journal of Sleep Research 2020.*

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