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IDENTIFICATION OF BIO AND DIGITAL MARKERS OF SLEEP,
FATIGUE AND ACTIVITIES OF DAILY LIVING IN
NEURODEGENERATIVE DISEASES

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ABSTRACT

Le misure esistenti della qualità del sonno, della sonnolenza diurna e della fatica, basate su misure soggettive e auto-risportate, presentano molte limitazioni per l'uso negli studi clinici e nello sviluppo terapeutico. Per la fatica non sono disponibili misure oggettive convalidate. Nel caso del sonno, la misura oggettiva della polisonnografia è costosa, scomoda per i partecipanti, richiede molto lavoro e non valuta l'impatto dei problemi del sonno. Inoltre, le misure esistenti non sono adatte all'uso in contesti ecologici. Le ricerche sulla fatica e sul sonno hanno dimostrato che questi sintomi sono associati a cambiamenti nella biofisiologia, nelle prestazioni neurocognitive e nei comportamenti fisici e sociali. Ad esempio, è stato dimostrato che la disfunzione autonoma è comune tra i pazienti affaticati in molte malattie neurodegenerative. Inoltre, i pazienti affaticati spesso riferiscono livelli ridotti di attività fisiche e di vita quotidiana, in particolare quelle che richiedono sforzi fisici da moderati a impegnativi. La riduzione dell'interazione sociale e delle funzioni a causa della fatica sono spesso identificate dai pazienti come impatti chiave di questo sintomo invalidante. È interessante notare che i cambiamenti nella biofisiologia, nelle prestazioni neurocognitive e nei comportamenti sono simili tra i pazienti con malattie neurodegenerative, indipendentemente dalle loro condizioni di base. Queste osservazioni suggeriscono che potrebbe esistere una "biosegnale" comune della fatica nelle condizioni croniche. È importante notare che, grazie ai progressi delle tecnologie digitali, è ora possibile monitorare alcuni di questi parametri in modo continuo o semi-continuo nell'ambiente di vita naturale. Tali progressi tecnologici aprono la possibilità di identificare biomarcatori oggettivi e digitali della fatica, della sonnolenza e della qualità del sonno. Questa tesi mira a trovare parametri oggettivi per la qualità del sonno, la sonnolenza e la fatica, valutati con dispositivi digitali mobili in pazienti affetti da malattie neurodegenerative, poiché i disturbi del sonno e la fatica sono prevalenti e onerosi in queste malattie. L'obiettivo è quello di esplorare

ulteriormente misure digitali selezionate per la qualità del sonno, la sonnolenza e la fatica che possono essere valutate anche con tecnologie digitali in ambiente domestico.

Rationale

Existing measures of sleep quality, daytime sleepiness and fatigue based on subjective, self-reported measures have many limitations for use in clinical studies and therapeutic development. Validated objective measures are not available for fatigue. In the case of sleep, the objective measure polysomnography is expensive, inconvenient for the participant, labour intensive and does not assess the impact of sleep problems. Furthermore, existing measures are unsuitable for use in ecological settings.

Research in fatigue and sleep has shown that these symptoms are associated with changes in biophysiology, neurocognitive performances as well as physical and social behaviours. For instance, autonomic dysfunction has been shown to be common among patients with fatigue in many neurodegenerative diseases.

Furthermore, fatigued patients often report reduced levels of physical activities and activities of daily living especially those requiring moderate to strenuous physical efforts. Reduced social interaction and function as a result of fatigue are often identified by patients as key impacts of this disabling symptoms. It is noteworthy that the changes in biophysiology, neurocognitive performances and behaviours are similar among patients with neurodegenerative diseases regardless of their underlying conditions. These observations suggest that there may be common fatigue “biosignature” in chronic conditions.

Importantly, with the advances in digital technologies, it is now possible to monitor some of these parameters continuously or semi-continuously in the natural living environment. Such technological advances raise the possibility of identifying objective bio and digital biomarkers of

fatigue, sleepiness and sleep quality.

This thesis aims to find objective parameters for sleep quality, sleepiness and fatigue, as assessed with mobile digital devices in patients suffering from neurodegenerative diseases, as sleep disturbances and fatigue are prevalent and burdensome in these diseases in order to further explore if these selected measures could be used in a clinical decision-making context.

1. RESEARCH BACKGROUND, OBJECTIVES AND AIMS

1.1 INTRODUCTION

The identification of biological and digital markers of fatigue and sleep in neurodegenerative diseases represents a critical frontier in modern healthcare research. Fatigue and sleep disturbances, as nonmotor symptoms, are pervasive in disorders like Parkinson's disease (PD), Huntington's disease, and other neurodegenerative disorders. These symptoms have profound implications for patient quality of life, disease progression, and overall healthcare outcomes. Recent advances in digital health technologies provide novel opportunities to monitor and manage these challenges, bridging gaps between research innovations and clinical applications.

Nonmotor symptoms, particularly autonomic dysfunction, behavioral changes, cognitive impairment, and sleep alterations, are increasingly recognized as core components of movement disorders. For example, sleep disturbances, a high-prevalence yet under-addressed issue in PD, are being studied using novel digital solutions like accelerometers, bed trackers, and AI-based respiratory monitors [1]. These technologies aim to provide cost-effective and accessible alternatives to polysomnography (PSG), the current gold standard for sleep assessment.

Idiopathic REM sleep behavior disorder (iRBD) has emerged as a significant area of interest due to its strong association with neurodegenerative conditions, particularly alpha-synucleinopathies like Parkinson's disease. Often considered a prodromal marker, iRBD provides a unique window for early detection and intervention in at-risk populations. [2] Leveraging digital tools for the continuous monitoring of REM sleep anomalies, including dream enactment behaviors and associated physiological changes, could facilitate earlier diagnosis and personalized therapeutic strategies. Integrating iRBD assessments into broader frameworks may further enhance our understanding of disease trajectories and improve patient care. (Study I, Study II and Study III)

The landscape of neurodegenerative diseases, especially movement disorders, is marked by complex motor and nonmotor challenges, with fatigue emerging as a particularly debilitating and under-assessed symptom. Fatigue often eludes comprehensive evaluation during routine clinical visits due to its fluctuating nature and dependence on environmental and physiological conditions. The advent of Mobile Health Technologies (MHT) and other digital solutions offers transformative opportunities for monitoring fatigue in real-world contexts. Traditional clinical scales, while valuable, are limited in capturing the transient and multifaceted aspects of fatigue that manifest during unsupervised daily activities. Digital devices bridge these gaps by providing continuous, context-rich data, enhancing our understanding of fatigue's role in disease progression and therapeutic responses [3, 4].

The versatility of digital technologies enables the simultaneous quantification of fatigue alongside other nonmotor parameters, such as cognitive and behavioral states, creating a holistic picture of disease burden. This multimodal approach is particularly vital for longitudinal monitoring, facilitating the tracking of disease trajectories and the efficacy of disease-modifying therapies [5]. For individuals with neurodegenerative diseases like Parkinson's disease (PD), these technologies offer critical insights into the daily impact of fatigue, allowing for the development of personalized care strategies that address its pervasive effects. (Study IV)

Beyond clinical utility, digital solutions address practical limitations of traditional assessment paradigms. Wearable and home-based technologies overcome barriers posed by time-constrained clinical settings, providing ecologically valid data that reflect real-world patient experiences. Studies have demonstrated that supervised and unsupervised assessments often yield complementary insights, emphasizing the need for hybrid approaches that integrate structured clinical evaluations with naturalistic monitoring [6]. (Study IV) However, challenges remain in standardizing data

collection and analysis, particularly in unsupervised settings where variability in activities and environmental factors can influence measurements.

In addition to monitoring fatigue, digital technologies also could play a crucial role in assessing the impact of neurodegenerative diseases on activities of daily living (ADLs).

Activities of daily living (ADLs) refer to essential tasks that individuals perform daily to maintain independence, such as eating, bathing, dressing, grooming, and managing household responsibilities. In individuals with neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and other movement disorders, the ability to perform these tasks is often significantly impaired due to a combination of motor and nonmotor symptoms.

In Parkinsonism particularly, motor symptoms such as rigidity, bradykinesia (slowness of movement), and tremors can directly interfere with a person's ability to carry out ADLs. [7] The difficulty in initiating movement or maintaining balance can make simple tasks like dressing or walking a challenging action. Nonmotor symptoms, such as fatigue, cognitive impairments, and mood disturbances, further complicate daily functioning. Fatigue, in particular, can reduce the person's energy levels, making it harder to engage in activities throughout the day, leading to dependency on caregivers.

Moreover, as these diseases progress, the severity of motor and nonmotor symptoms tends to increase, further impairing the ability to carry out ADLs independently. For patients with more advanced stages of neurodegenerative diseases, the loss of independence in ADLs is often one of the most distressing aspects of the disease, affecting their quality of life.

Digital technologies offer a promising approach to track the impact of neurodegenerative diseases on ADLs. (Study V) These technologies can capture real-time data on mobility that directly influence a person's ability to engage in daily tasks. By providing continuous, objective data, digital health tools can help clinicians monitor functional decline over time and identify early signs of worsening

symptoms. This allows for personalized care plans that focus not only on treating the symptoms but also on maintaining or improving independence in ADLs, ensuring that patients can continue to participate in daily activities for as long as possible.

1.2 AIMS

This doctoral thesis aims to identify and validate biological and digital markers of fatigue and sleep disturbances in neurodegenerative diseases. The integration of multimodal digital assessment technologies, such as wearable devices, AI-driven algorithms, and mobile health tools, offers unprecedented opportunities for early diagnosis, personalized interventions, and real-time monitoring in clinical and non-clinical settings. By exploring the intersection of established clinical methodologies and emerging digital health innovations, this research seeks to bridge the gap between pilot studies and practical, individualized patient care.

The specific objectives of this research are:

1. To investigate the feasibility and reliability of digital tools for assessing fatigue and sleep disturbances in neurodegenerative diseases.
2. To identify key bio and digital markers associated with these nonmotor symptoms and their progression.
3. To evaluate the potential of multimodal approaches in improving diagnostic accuracy
4. To provide a framework for integrating these markers into clinical practice, enhancing patient-centered care through personalized, technology-driven solutions.

By synthesizing insights from cutting-edge digital health research and addressing current clinical limitations, this thesis aims to advance the understanding and management of fatigue and sleep in neurodegenerative diseases, setting the stage for future innovations in the field.

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1.4 SUMMARY OF THE PHD PROJECT AND RELATED RESULTS

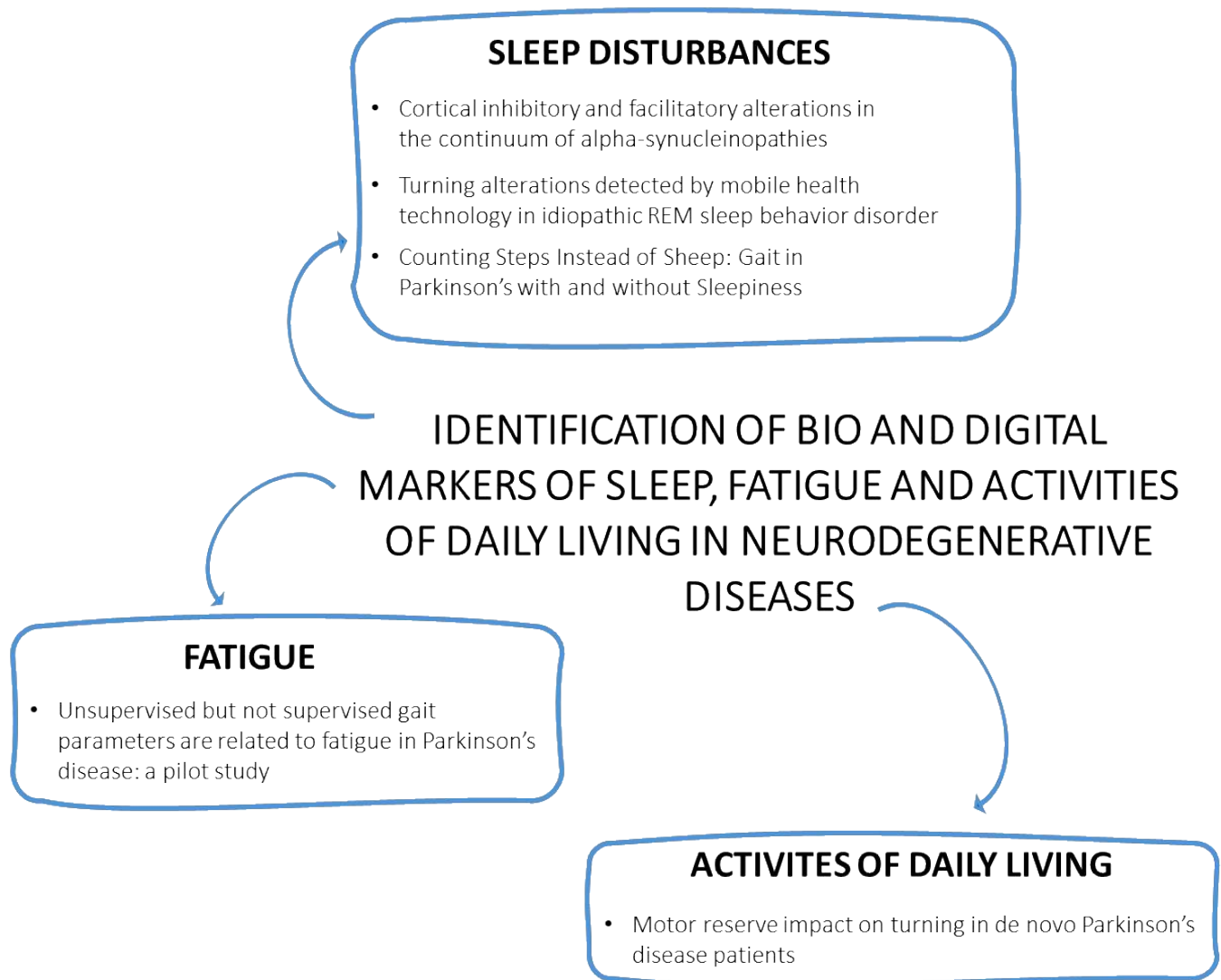


Figure 1. Schematic representation of the PhD project's topics with relative studies (listed below each topic.

SLEEP DISTURBANCES

Summary

Cortical Inhibitory and Facilitatory Alterations in the Continuum of Alpha-Synucleinopathies

The first abstract explores the role of cortical inhibitory and facilitatory alterations in neurodegenerative diseases, focusing on the continuum of alpha-synucleinopathies, including idiopathic REM sleep behavior disorder (iRBD), Parkinson's disease (PD), and dementia with Lewy bodies (DLB). Sleep disturbances are common in these conditions, often presenting in the

prodromal stages, such as in iRBD, which is considered a precursor to PD. The study employed transcranial magnetic stimulation (TMS) to assess alterations in GABAergic, glutamatergic, and cholinergic circuits, revealing significant impairments in both iRBD and DLB patients, compared to healthy controls. Specifically, iRBD patients exhibited reduced short-latency afferent inhibition (SAI), indicating early cholinergic dysfunction that mirrors the dysfunction seen in DLB. These findings underline the importance of identifying sleep disturbances early in the disease process, as they may serve as indicators of broader neurophysiological changes. Using Mobile Health Technologies (MHT) for monitoring these early signs could provide valuable insights into the progression of alpha-synucleinopathies and facilitate early intervention before the emergence of more pronounced motor symptoms.

Subtle Motor Impairments in Prodromal Stages: iRBD as an Early Indicator

The second study focuses on subtle motor impairments in individuals with idiopathic REM sleep behavior disorder (iRBD), a condition at high risk of developing Parkinson's disease. Using mobile health technology (MHT), the study assessed turning performance and detected subtle motor changes in iRBD individuals without clinical Parkinsonism. Compared to healthy controls, iRBD participants demonstrated longer turn durations and lower angular velocities, particularly at normal speed. These findings suggest that MHT could be a valuable tool in identifying early signs of motor impairments and offer a way to track the early stages of disease progression. Such objective monitoring could prove essential for detecting the onset of symptoms that affect daily activities, providing a foundation for future studies tracking motor progression in prodromal Parkinson's disease.

Impact of Sleepiness on Gait in Parkinson's Disease: Insights from Real-Life Monitoring

The third study investigates the impact of sleepiness on gait performance in drug naïve Parkinson's disease (PD) patients, highlighting the critical role that sleep-related issues play in daily functioning. Sleep disturbances, a common and disabling symptom of PD, can significantly impair motor function, including gait. This study utilized Mobile Health Technologies (MHT) to assess gait under supervised conditions, revealing significant differences between PD patients with and without sleep problems. PD patients with sleepiness demonstrated greater gait problems compared to those without. The correlation between sleepiness severity and changes in gait parameters emphasizes the importance of addressing sleep issues in PD patients.

FATIGUE

Summary

Fatigue and Its Influence on Gait in Real-Life Settings

The fourth study investigates the impact of fatigue on gait performance in Parkinson's disease (PD) patients, using mobile health technologies (MHT) to assess gait under both supervised and unsupervised conditions. The study found that, in real-life scenarios, patients with fatigue (PD-F) exhibited significantly slower, more asymmetric gait compared to those without fatigue (PD-N). These differences were particularly pronounced under unsupervised conditions, showing that fatigue impairs daily gait in ways that might not be detected in a clinical setting. The correlation between fatigue severity and gait alterations suggests that fatigue is a critical factor influencing mobility and daily activities in PD patients. By incorporating MHT in real-world environments, clinicians can more accurately assess and address fatigue-related gait disturbances, leading to better tailored interventions to improve daily functioning.

ACTIVITIES OF DAILY LIVING

Summary

Motor Reserve (MR) and Its Impact on Daily Activities in Parkinson's Disease

The fifth study examines the concept of motor reserve (MR) and its role in enhancing motor performance in individuals with Parkinson's disease (PD). The study explored the relationship between physical activity, motor reserve, and motor function, finding that higher MR was associated with better turning efficiency in drug-naïve PD patients. MR, which reflects the ability to adapt to motor decline through lifelong physical activity, appeared to modulate the motor impairments in PD patients. The results suggest that maintaining a high motor reserve through physical activity may help preserve daily motor function and improve the efficiency of common daily tasks, particularly in the early stages of PD. This highlights the importance of physical activity not only for overall health but also for enhancing the quality of daily activities and counteracting the motor difficulties associated with PD.

This structure creates a strong narrative connecting the concepts of motor reserve and its role in maintaining daily activities, with the progression from assessing motor performance in early disease stages (iRBD) to long-term motor decline in PD. It ties together the relevance of MHT in daily life monitoring, ensuring that the daily impact of symptoms and interventions is central to the thesis.

2. RESEARCH ACTIVITY

2.1 SLEEP DISTURBANCES

A) Cortical inhibitory and facilitatory alterations in the continuum of alpha-synucleinopathies

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INTRODUCTION

Alpha-synucleinopathies are neurodegenerative disorders characterized by a wide range of motor and non-motor symptoms. The clinical course of alpha-synucleinopathies is heterogeneous, and different pathophysiological processes may be involved at different disease stages. Among these, alterations in inhibitory and facilitatory neuronal circuits, particularly early in the disease course, are increasingly recognized as critical for understanding the onset and progression of the disease.

[1-3]

Idiopathic rapid eye movement (REM) sleep behaviour disorder (iRBD), a parasomnia characterized by the enactment of vivid, often unpleasant dreams during REM sleep, is widely regarded as a prodromal symptom of Parkinson's disease (PD) and Lewy Body disease (DLB). Evidence suggests that iRBD may precede the classic extrapyramidal features of PD/DLB by several years, offering an important window into the early pathophysiological processes of these disorders. [4]

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that can detect changes in the activity of specific neurotransmitter circuits *in vivo*. [5] TMS has been increasingly used to investigate cortical excitability and plasticity in several neurological disorders,

including PD and DLB. Several studies have suggested that cortical disinhibition, reflected by changes in short interval intracortical inhibition (SICI), may represent an early and consistent marker of PD. SICI changes have been observed across the continuum of alpha-synucleinopathies, from early to late disease stages [6], although there is significant variability and a notable influence of treatment across studies [7]. However, the application of TMS to study prodromal alpha-synucleinopathies, particularly in patients with iRBD, has been limited. Early TMS studies in individuals with iRBD have reported abnormalities in short latency afferent inhibition [8, 9], suggesting an impairment in cholinergic circuitry. These findings support the hypothesis that iRBD, often associated with an increased risk of conversion to DLB and cognitive decline in alpha-synucleinopathies [10], may be driven by cholinergic dysfunction [11]. Despite these insights, a comprehensive understanding of neurotransmitter circuit alterations in alpha-synucleinopathies, especially during prodromal stages, remains elusive.

To address this gap, we aimed to explore changes in GABAergic, glutamatergic, and cholinergic circuits in patients with PD, including drug-naïve PD, patients with iRBD and patients with DLB. By leveraging the capabilities of TMS, we sought to uncover novel insights into the pathophysiological underpinnings of alpha-synucleinopathies, with crucial implications for early detection, prognosis, and management.

To date, only a few studies have used TMS to investigate iRBD. An initial study identified abnormalities in short-latency afferent inhibition (SAI), suggesting cholinergic impairment in these patients. This finding was supported by a subsequent study [12], which included both iRBD and PD patients. In the present study, we applied TMS to patients with alpha-synucleinopathies to determine whether cortical inhibitory and facilitatory mechanisms might support the idea that iRBD could serve as a valuable model for characterizing the continuum of alpha-synucleinopathies.

METHODS

Participants and clinical assessment

This prospective cross-sectional study enrolled consecutive patients from the outpatient Movement Disorder clinic, Neurology Unit at the University of Brescia, as well from the Sleep disorder Centers of ASST Spedali Civili of Brescia, Italy, between January 2023 and May 2024. The research protocol was approved by the Ethics Committee of the Brescia Hospital, Brescia, Italy (DMA study, NP 1471, with the latest version on April 22, 2022). Written informed consent was obtained from all participants.

Individuals with established iRBD were included based on the following criteria: i) history of dream-enacting behaviour; ii) polysomnography (PSG) proven REM sleep with sustained electromyographic (EMG) activity; (iii) absence of known pathological neurologic conditions; iv) lack of motor and cognitive complaints, v) normal magnetic Resonance imaging (MRI) and vi) absence of other sleep disorders (*e.g.*, restless legs syndrome, periodic limb movements, obstructive sleep apnea syndrome, insomnia, narcolepsy, sleep-wake rhythm disorders), medical disorders, medications, or substance abuse [1]; vii) no gait abnormalities on standard neurological examination.

Patients with clinically established PD or DLB were also included [13]. The following exclusion criteria were applied: i) symptoms or features suggesting other atypical parkinsonism [13]; ii) other neurological disorders or medical conditions potentially associated with gait disturbances; iii) cortical cerebrovascular diseases or other central nervous system diseases detected by MRI; iv) diagnosis of epilepsy; v) requirement of walking aids; vi) normal dopamine uptake in nigrostriatal imaging. Additional exclusion criteria were: age < 18 years; presence or history of any psychiatric diseases (*e.g.*, depressive, bipolar, or psychotic disorders); presence of impulse control disorder; acute or non-compensated illnesses; presence of clinical or drug-associated conditions causing

cognitive or mood dysfunction; use of psychoactive drugs or medications capable of modulating brain excitability [14], including cholinesterase inhibitors; any contraindications for MRI or TMS. Each participant underwent a standardized medical and neurological examination, including the Movement Disorder Society-Unified Parkinson Disease Rating Scale (MDS-UPDRS) and Hoehn and Yahr stage (H&Y) assessment [15]. Non-motor function was evaluated with the Non-Motor Symptoms Scale for Parkinson's disease (NMSS) [16]. Other specific scales were also used: the REM sleep behavior disorder screening questionnaire (RBDSQ) to assess RBD [17], the Sniffing Sticks test [18] for olfactory function [19], the Montreal Cognitive Assessment (MoCA) for cognitive function [20], the Neuropsychiatric Inventory (NPI) for neuropsychiatric symptoms [21], and the Beck's Depression Inventory (BDI) for mood assessment [22]. DLB patients were classified as having low cholinergic activity when the mean values of SAI were three standard deviations below those of HC values. RBD symptoms were assessed using the RBDSQ, with a score of 7 points or higher considered indicative of RBD. [17]

Transcranial magnetic stimulation

TMS was performed with a figure-of-eight coil (each loop diameter 70 mm) connected to a Magstim Bistim² system (Magstim Company, Oxford, UK) as previously reported [11]. Motor evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle (FDI) through surface Ag/AgCl electrodes placed in a belly-tendon montage and acquired using a Biopac MP-150 electromyograph (BIOPAC Systems Inc., Santa Barbara, CA, USA).

To locate the precise representation of the target muscle on the contralateral primary motor cortex, the TMS coil was positioned approximately 4 cm laterally and 2 cm anteriorly to Cz, tangentially on the scalp with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The "hot spot" was defined as the point in which magnetic stimulation resulted in the maximum motor evoked potential (MEP) amplitude with the minimum stimulator intensity. To

obtain this, stimulator intensity was increased from 35% of the maximal stimulator output (MSO) in 5% steps until MEPs with an approximately 0.5-1 mV amplitude could be recorded. The coil was then moved in 0.5 cm steps medially, laterally, posteriorly and anteriorly while evoking 3 MEPs at each site. This was performed until the site in which the largest MEPs could be located, which was marked with a felt tip pen on the scalp to ensure constant placement of the coil throughout the experiment [23].

Resting motor threshold (RMT) was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 50 μ V in 5 out of 10 consecutive trials during complete muscle relaxation, which was controlled by visually checking the absence of EMG activity at high-gain amplification. RMT was determined according to the relative frequency method, in which we started at a stimulus intensity of 35% MSO with the coil placed over the motor “hot spot”, and stimulus intensity was gradually increased in steps of 5% MSO until TMS consistently evoked MEPs with peak-to-peak amplitudes of >50 μ V in each trial for RMT. Thereafter, stimulus intensity was gradually lowered in steps of 1% MSO until there were less than 5 positive responses out of 10 trials.

Short interval intracortical inhibition (SICI), intracortical facilitation (ICF) and short latency afferent inhibition (SAI) were studied using a paired-pulse protocol, employing a conditioning-test design. For all paradigms, the test stimulus (TS) was adjusted to evoke a MEP of approximately 1 mV peak-to-peak amplitude.

For SICI and ICF, the conditioning stimulus (CS) was adjusted at 70% of the RMT, employing multiple interstimulus intervals (ISIs), including 1, 2, 3 ms for SICI and 7, 10, 15 ms for ICF[24, 25] .

SAI was evaluated employing a CS consisting of a single pulse (200 μ s) of electrical stimulation at the right median nerve at the wrist, using a bipolar electrode with the cathode positioned proximally, at an intensity sufficient to evoke a visible twitch of the thenar muscle. Different ISIs

were implemented (0, +4 ms), which were fixed relative to the peak latency of the N20 component of the somatosensory evoked potential of the median nerve [26].

For each ISI and for each protocol (SICI, ICF, and SAI), 10 different paired CS-TS and control TS were delivered in all participants in a pseudo randomized sequence, with an inter-trial interval of 5 secs ($\pm 10\%$).

The conditioned MEP amplitude, evoked after delivering a paired CS-TS, was expressed as percentage of the mean control MEP amplitude. Stimulation protocols were conducted in a randomized order. Audio-visual feedback was provided to ensure muscle relaxation during the entire experiment and trials were discarded if EMG activity exceeded 25 μV prior to TMS stimulus delivery. Less than 5% of trials were discarded for each protocol. All the participants reached complete muscle relaxation; in the case the data was corrupted by patient movement, the protocol was restarted, and the initial recording was rejected.

The operators who administered TMS were blinded to the subjects' status; standardized TMS procedures were employed for all participants and stimuli were delivered in a randomized sequence, thus reducing possible biases in TMS recordings.

Statistical analysis

Comparison of clinical characteristics was carried out using Mann-Whitney-U test or chi-square test of independence, as appropriate. Neurophysiological parameters were compared by means of two-way mixed ANOVA with ISI as within-subjects factor and GROUP as between-subjects factor. When a significant main effect was reached, *post-hoc* tests with Bonferroni correction for multiple comparisons were conducted to analyze group-differences at respective ISIs. Mauchly's test was used to check for assumption of sphericity, while Greenhouse-Geisser epsilon was used to correct in case of sphericity violation. Statistical significance was assumed at $p < 0.05$. Data analyses were carried out using SPSS 26.0 software.

RESULTS

One-hundred and thirty participants were included in the study, namely 75 with PD, (45 drug-naïve and 30 on dopaminergic treatments), 15 with iRBD, 20 with DLB patients and 20 healthy controls (HC) (the flow chart of included participants is shown in Supplementary Table 1). All subjects completed all TMS procedures without discomfort or adverse effects. Although some patients reported mild sleepiness, none became drowsy during the stimulation sessions, and no prompting was required to maintain vigilance. The clinical and demographic characteristics of the participants are summarized in Table 1. There were no significant differences in age or disease severity between the patient groups; however, sex distribution differed across groups, and thus sex was included as nuisance variable in the analyses. Both PD and DLB exhibited similar total MDS-UPDRS-III score, whereas DLB patients showed higher MDS-UPDRS I and MDS-UPDRS II compared to the PD groups. Our data also revealed higher RBDSQ scores in iRBD patients compared to both drug-naïve and treated PD patients. In agreement with this, 20 out of 45 drug-naïve PD patients had RBDSQ scores above the cutoff values, while 10 out of 30 treated PD patients exceeded the cutoff.

Figure 1 shows the results of paired-pulse TMS. Repeated-measures ANOVA performed on SICI-ICF revealed a significant ISI $\times$ Group interaction for SICI ($F(11.52,575.98)=65.23$, $p=0.009$, partial $\eta^2=0.591$) and a significant ISI $\times$ Group interaction for SAI ($F(9.11,248.13)=7.461$, $p=0.000$, partial $\eta^2=0.215$). *Post-hoc* comparisons revealed significant differences between PD and HC at ISIs 1, 2, and 3 ms, between HC and iRBD at ISIs 2 and 3 ms, and between HC and DLB at ISIs 2 and 3 ms (all $p<0.05$). Additionally, lower values of SICI were observed in PD compared to DLB patients at ISI 3 ($p<0.05$).

For ICF, we observed a significant difference between DLB and all other groups at ISI 7, 10, and 15 ms, between HC and iRBD at ISI 10 ms, and between HC and PD at ISI 10 and 15 ms (all $p<0.05$).

Analysis at the single-subject level in iRBD patients showed values either similar to HC (9 subjects, 60% of total) or below the value of HC (6 subjects, 40% of total) (Supplementary Table 2).

iRBD significantly differed from HC and PD at ISIs 0, +4 ms for SAI (all $p < 0.001$), and DLB showed significant differences from PD and HC at ISIs 0, +4 ms, though no differences were found between HC and PD or between iRBD and DLB. Subanalysis comparing PD patients with and without RBD symptoms showed no differences (Supplementary Table 3). No correlation between SICI/SAI alterations and clinical variables (including MDS-UPDRS-III, MoCA, NPI scores) was observed in the entire sample or within the separate groups.

DISCUSSION

This study explored intracortical circuits which indirectly and partially reflect changes in GABAergic, glutamatergic, and cholinergic circuits in the prodromal and early stages of alpha-synucleinopathies using TMS, aiming to investigate whether cortical alterations in iRBD patients could serve as biomarkers for the prodromal stages of PD and DLB. The study confirmed significant alterations in GABAergic and glutamatergic circuits in both drug-naïve and treated PD patients, as well as in DLB patients, supporting the idea that these changes are a fundamental pathophysiological feature of alpha-synucleinopathies [6]. In contrast, cholinergic alterations were observed primarily in iRBD and DLB patients, distinguishing them from controls and PD patients. These findings enhance our understanding of the role of neurotransmitter circuits across the continuum of alpha-synucleinopathies, revealing that both inhibitory and excitatory circuits are impacted from the early stages of the disease.

It is noteworthy that glutamatergic/GABA alterations were observed in patients with PD who had not yet received pharmacological treatment, in line with previous large-scale studies focused on drug-naïve PD patients [27-29]. Additionally, the presence of such changes in the prodromal stage, as represented by the iRBD group, suggests that these alterations may emerge early in the disease and are likely not associated with the presence of motor symptoms, but rather with the underlying neurodegenerative nigrostriatal dysfunction. Of note, this study found that the pattern of alterations in intracortical facilitation was varied in iRBD patients. Specifically, these patients exhibited a wider range of variation (as assessed by standard deviation), with values either similar to or below those of HCs. The individual variability observed in ICF in iRBD is of particular interest, as it may be associated to different disease progression patterns or ongoing compensatory mechanisms [4] [30]. Thus, participants with a low glutamatergic activity may be at increased risk

for developing a DLB, whereas those with ICF values similar to HCs may reflect a condition more consistent with PD, according to the SICI-ICF data that we extracted for DLB and PD patients. Furthermore, the study noted a significant reduction in SAI in iRBD and DLB compared to both PD patients and healthy controls. This finding, consistent with previous reports, suggests a widespread cholinergic neurophysiological alteration in individuals with iRBD. This warrants further investigation, as it may have implications for identifying individuals with distinct neurophysiological profiles and varying risks of developing cognitive impairment within the spectrum of PD and DLB. The pathophysiology of long-standing iRBD has been indeed associated with cholinergic alterations, as revealed by other technique, including imaging studies [31]. It is noteworthy that the study only found a trend toward dysfunction in PD patients who developed RBD during the disease, which contrasts with earlier reports on PD [12]. However, these findings are consistent with recent data suggesting a different pathophysiology underlying RBD in early PD compared to that observed in subjects with long-term idiopathic RBD [32].

The identification of glutamatergic/GABA and cholinergic signatures in the prodromal and early phases of alpha-synucleinopathies could have significant implications for better understanding the heterogeneous progression and potential compensatory mechanisms associated with iRBD, as well as the different conversion and progression patterns, an area that remains an open question for researchers and clinicians. Despite the importance of these findings, it is crucial to acknowledge several limitations, including the cross-sectional design, which allows for the identification of associations but not causality. Ongoing longitudinal studies are thus warranted to confirm and extend these findings, and to evaluate the correlation between individual neurophysiological changes and progression patterns at the single subject level.

Additionally, while TMS is a valuable tool for studying cortical excitability, it does not directly measure neurotransmitter activity. Future studies using the combination of neurophysiology and

neuroimaging techniques may complement and extend these findings by elucidating the complex relationship between cholinergic/glutamatergic changes and dopaminergic/serotonergic alterations *in vivo* [33, 34].

In summary, this study adds to the growing body of evidence emphasizing the importance of neurotransmitter circuitry alterations in the development of PD. It highlights the potential of non-invasive brain stimulation techniques, such as TMS, in revealing these changes and enhancing our understanding of the disease. Looking ahead, these findings present promising opportunities for early detection, individual stratification, and prognosis of PD. Furthermore, these neurophysiological alterations may represent targets for innovative therapeutic strategies.

Table 1. Clinical and demographic characteristics of included participants

	HC	iRBD	Drug-naïve PD	Treated PD	DLB	<i>p</i> -value
Participants [n]	20	15	45	30	20	
Age [years]	67.1±12.5	71.4±6.2	68.9±7.8	67.7±9.3	76.3±13.6	0.363 ^c
Sex [% male]	45	87	73	67	80	0.002^a
Disease duration [years]	-	5.7±2.6	1.7±1.5	5.5±3.5	3.6±1.5	0.000^b
MDS-UPDRS I [points]	-	4.6±4.8	6.1±4.5	5.1±4.9	11.4±6.2	0.011^c \$ #
MDS-UPDRS II [points]	-	1.0±1.6	5.0±4.0	4.2±4.7	7.0±5.4	0.011^c §
MDS-UPDRS III [points]	-	-	18.3±9.0	16.5±9.6	19.3±16.4	0.233 ^b
H&Y stage	-	-	1.4±0.7	1.8±1.3	2.4±0.9	0.260 ^b
MoCA [points]	26.8±1.8	26.0±1.4	26.0±2.3	26.5±1.7	18.8±9.3	0.382 ^c
BDI [points]	1.3±2.1	5.9±2.5	5.0±5.5	6.7±4.0	6.8±4.0	0.001^c \$ ^ Ω
NPI [points]	2.0±2.0	7.3±3.6	7.3±4.8	4.0±2.7	6.2±3.4	0.001^c \$ ^ Ω
NMSS [points]	7.3±5.9	21.0±13.2	18.5±21.9	20.0±26.0	23.8±15.2	0.001^c \$ ^ Ω
Sniffing Test [points]	8.1±2.8	3.7±2.5	4.2±2.9	3.1±2.8	4.8±4.0	0.009^c \$ ^ Ω
ESS [points]	3.0±2.1	7.4±3.6	4.2±4.1	4.3±3.6	3.4±3.2	0.018^c \$ §
RBDSQ [% positive]	5	80	44	33	60	0.001^c * \$ ^
LEDD	-	-	0	466.7±254.0	188.5±131.6	0.000^c #

a. Chi-square test

b. Mann-Whitney-U test

c. with Bonferroni Correction for 4 comparisons (* iRBD vs PD; § iRBD vs DLB; # PD vs DLB, \$ HC vs iRBD, ^ HC vs DLB, Ω HC vs PD)

MDS-UPDRS, Movement Disorder Society Unified Parkinson's disease Rating scale; H&Y, Hoehn and Yahr stage; MoCA, Montreal Cognitive Assessment; BDI, Beck Depression Inventory; NPI, neuropsychiatric inventory; ESS, Epworth Sleepiness Scale; RBDSQ, RBD screening questionnaire; LEDD, levodopa equivalent daily dose.

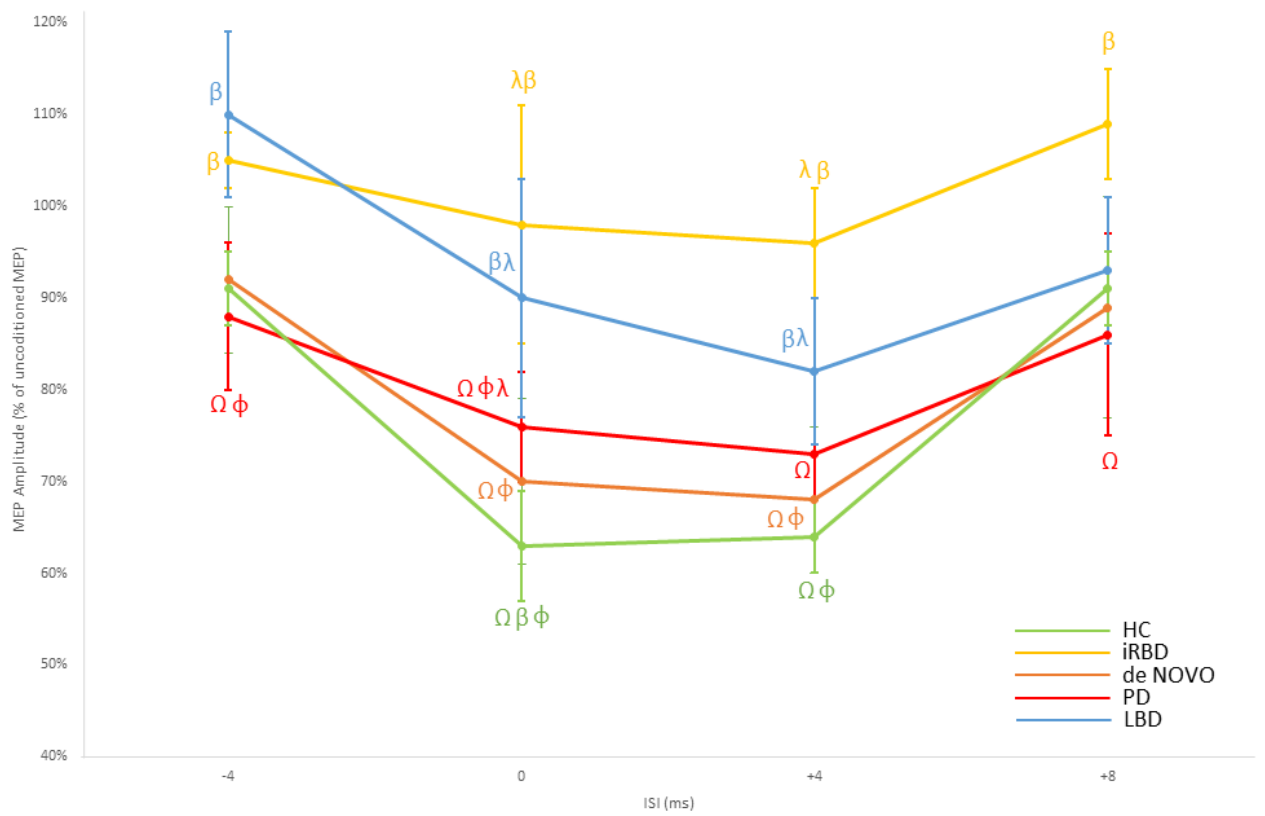
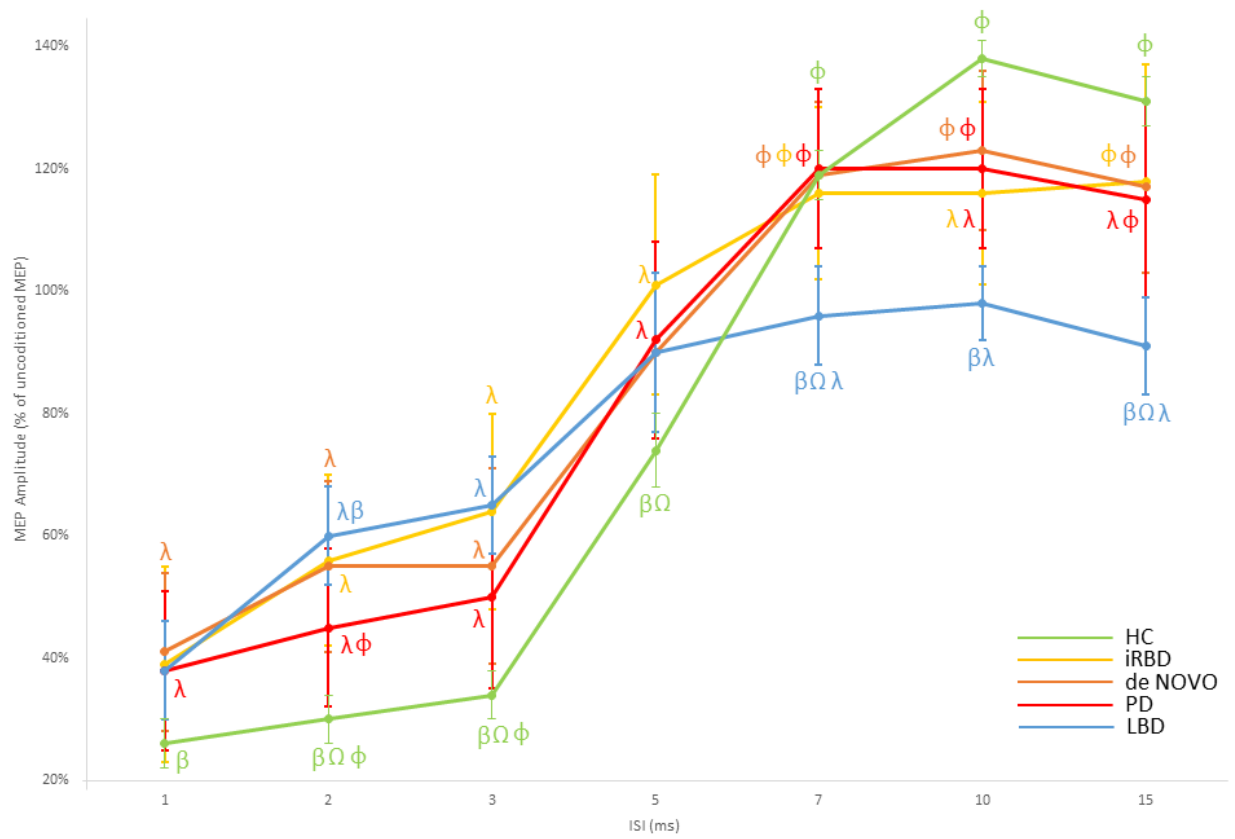
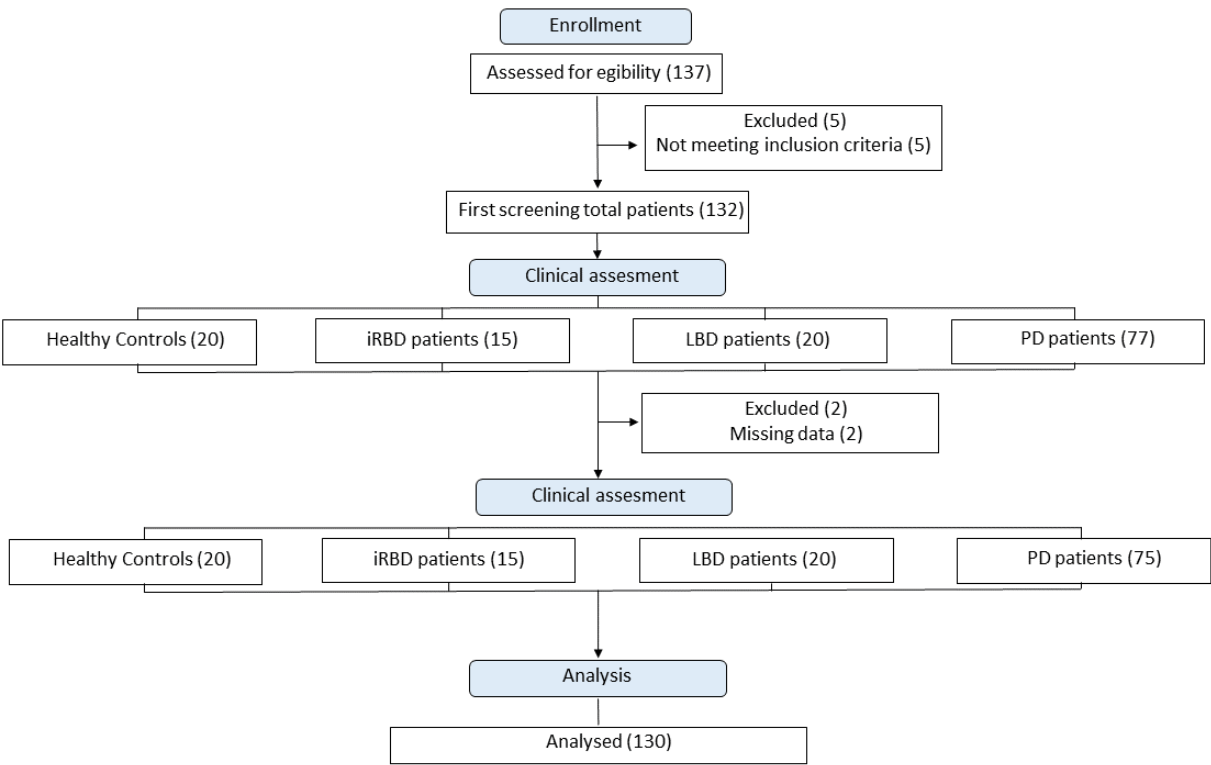


Figure 1. Neurophysiological parameters in SICI-ICF (top) and SAI (bottom) at different ISIs in iRBD, naïve, treated PD, DLB and HC. Data are plotted as a ratio to the unconditioned MEP amplitude. Error bars for each ISI represent the standard error of the mean. SICI-ICF = short interval intracortical inhibition; ISI = interstimulus interval; MEP = motor evoked potential; Ω $p < 0.05$ vs iRBD, β $p < 0.05$ vs PD, ϕ $p < 0.05$ vs DLB, λ $p < 0.05$ vs HC

Supplementary Table 1. Workflow of the study.



Supplementary Table 2. Single subject values of ICF in iRBD patients.

	mean ICF 7,10,15 ms	status
Subject 1	1.250137	↑
Subject 2	1.446650	↑
Subject 3	1.703221	↑
Subject 4	0.801301	↓
Subject 5	0.904537	↓
Subject 6	1.481813	↑
Subject 7	1.273451	↑
Subject 8	0.998888	↓
Subject 9	0.893994	↓
Subject 10	0.641886	↓
Subject 11	1.396356	↑
Subject 12	1.573220	↑
Subject 13	1.296356	↑
Subject 14	1.381813	↑
Subject 15	0.930137	↓

↑ Value similar to HC; ↓ Value three standard deviations below HC

Supplementary Table 3. Mean TMS parameters in the different groups. Neurophysiological characteristics are expressed as mean $\pm$ standard deviation; rMT is expressed as ratio of the MSO; SICI-ICF and SAI are expressed as ratio of the mean MEP amplitude related to the control MEP. a. Bonferroni Correction for 4 comparisons (* iRBD vs PD; § iRBD vs DLB; # PD vs DLB, \$ HC vs iRBD, ^ HC vs DLB, Ω HC vs PD)

	iRBD	Drug-naïve PD	Treated PD	DLB	HC	p-value
RMT(%MSO)	43.5 $\pm$ 9.0	44.2 $\pm$ 7.1	42.9 $\pm$ 7.2	43.9 $\pm$ 3.9	44.5 $\pm$ 7.9	0.575 ^a
mean SICI 1, 2, 3 ms	0.55 $\pm$ 0.27	0.51 $\pm$ 0.24	0.42 $\pm$ 0.22	0.54 $\pm$ 0.24	0.29 $\pm$ 0.12	0.004^a ^\$
mean ICF 7, 10, 15 ms	1.15 $\pm$ 0.31	1.18 $\pm$ 0.21	1.14 $\pm$ 0.23	0.96 $\pm$ 0.18	1.27 $\pm$ 0.09	0.245 ^a
mean SAI 0, +4 ms	0.97 $\pm$ 0.10	0.68 $\pm$ 0.15	0.73 $\pm$ 0.14	0.86 $\pm$ 0.13	0.63 $\pm$ 0.19	0.001^a *\$^#

RMT = resting motor threshold; % MSO = percentage of maximal stimulator output; ICF, intracortical facilitation; SICI = short interval intracortical inhibition; SAI = short latency afferent inhibition; MEP = motor evoked potential.

Supplementary Table 4. Comparison between PD patients with and without RBD symptoms using RBDSQ cutoff values

	PD-RBD+	PD-RBD-	p-value
Participants [n]	31	44	

SAI, 0 ms	0.73±0.17	0.71±0.17	0.865 ^a
SAI, +4 ms	0.72±0.15	0.61±0.23	0.105 ^a

a. Mann-Whitney-U test

PD-RBD+ Parkinson's disease patients with RBD symptoms; PD-RBD- Parkinson's disease patients without RBD symptoms; SAI = short latency afferent inhibition; MEP = motor evoked potential.

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B) Turning alterations detected by mobile health technology in idiopathic REM sleep behavior disorder

Zatti C, Pilotto A, Hansen C, **Rizzardi A**, Catania M, Romijnders R, Purin L, Pasolini MP, Schaeffer E, Galbiati A, Ferini-Strambi L, Berg D, Maetzler W, Padovani A. Turning alterations detected by mobile health technology in idiopathic REM sleep behavior disorder. NPJ Parkinsons Dis. 2024 Mar 18;10(1):64. doi: 10.1038/s41531-024-00682-6. PMID: 38499543; PMCID: PMC10948811.

INTRODUCTION

Idiopathic Rapid Eye Movement sleep behavior disorder (iRBD) is the diagnosis associated with the highest risk of developing an alpha-synucleinopathy, particularly Parkinson's disease (PD) and dementia with Lewy bodies (DLB)(1). The overall risk of conversion within 10-12 years is 60-80%(1). Clinically, iRBD is defined by abnormal dream enacting behaviours with a persisting of muscle tone during the REM phases of sleep, in which the muscle tone is normally markedly reduced. The gold standard for the diagnosis and evaluation of iRBD is video-polysomnography (PSG)(2). Therefore, iRBD represents an important model to study disease progression in prodromal phases, when no or only subtle motor features are present (3). It was already shown that iRBD can be associated with mild Parkinsonian signs, such as isolated upper-limb bradykinesia, tremor, and reduced arm-swing. However, to our best knowledge, no study to date investigated turning performance in prodromal PD phases. We therefore aimed at evaluating turning in persons with iRBD using mobile health technology.

RESULTS

25 consecutive iRBD patients, 75 age-matched drug-naïve patients with parkinsonism, and 65 age-matched controls were screened. 2 iRBD were excluded due to presence of comorbid obstructive apnoea and alcohol abuse. From the group of drug-naïve parkinsonism, 15 participants were excluded due to signs for atypical parkinsonism (n=4), isolated tremor (n=8), drug-induced parkinsonism (n=2) and severe leukoencephalopathy at MRI (n=1).

The demographic characteristics and clinical features of the participants are shown in table 1. PD and iRBD showed similar prevalence and severity of non-motor features. As expected by applying the exclusion criteria, none of the iRBD patients exhibited subthreshold parkinsonism (mean 2 ± 2 points, non-significant to controls), as defined by prodromal MDS criteria, that is a MDS-UPDRS-III

score >6 excluding postural and action tremor(14). No iRBD participant exhibited cognitive impairment.

Mobile Health technology timed up and go assessment

Table 2 shows the total time of TUG and the digital parameters related to turning in the three groups. The overall duration of TUG tests was longer in PD compared to controls, with no differences between RBD and controls at normal and fast speed.

In both normal and fast speed assessments, drug-naïve PD but not iRBD showed significantly longer turning duration and slower turning speed compared to controls. The digital analyses on normal speed turning revealed lower mean and peak angular velocity in iRBD compared to controls (respectively $p=0.006$ and $p<0.001$ with absolute differences of -15.0 ± 3.4 and -41.9 ± 7.7). Compared to PD, iRBD showed a similar duration of turns but higher mean and peak angular velocity.

At fast speed, PD showed longer turning duration and lower angular velocity (peak and mean) compared to controls ($p<0.001$ with absolute differences of 0.7 ± 0.1 , -54.0 ± 5.5 and -20.4 ± 2.4 respectively). Conversely, iRBD did not differ significantly from controls in turning duration, mean and maximum velocities.

The variation percentages of TUG duration were significantly greater in iRBD in comparison to controls and PD; a similar (not significant) trend of percentage of variation was observed in iRBD for mean and peak angular velocities- which resulted higher in this group compared to PD and controls. The analysis of clockwise and counter-clockwise tests separated are present as supplementary material and showed similar pattern of distribution from PD, iRBD and PD – with iRBD vs controls differences more evident for left vs right turning (supplementary Table 1). Clockwise and counter-clockwise parameters in people with Parkinson's disease (PD) in which the left side is most affected

(PD Left side) and in which the right side is most affected (PDRight side) did not show significant differences (Supplementary Table 2).

DISCUSSION

The group of iRBD is at high risk of conversion to alpha-synucleinopathy and is thus target of several studies focusing on markers to track motor progression over time (3, 15, 16) (Supplementary Table 3). Still, the relevance of subtle motor impairment and its progression over time in prodromal PD stages are still not well understood. Findings showed that the standard measurement of time for completing the TUG test were able to differentiate PD but not iRBD from matched controls. Conversely, the digital assessment of turning detected important differences between iRBD and controls, even in absence of any relevant parkinsonian signs at normal examination. Our analyses were indeed able to detect significant subclinical differences between groups, below the minimal clinically significant value for the time duration of TUG, set at 3.5s (17). The digital assessment analyses were focused on turning duration, mean and peak angular velocity, which are standard parameters already demonstrated to differentiate PD from controls in different cohorts(10). Turning performances were evaluated in TUG performed separately at normal and fast speed, as task performed at higher speed might be more sensitive compared to usual speed.

In TUG performed at normal speed, iRBDs exhibited increased duration of turning and decreased mean and peak angular velocity compared to controls, at less degree compared with PD. These alterations - typically associated with parkinsonism (10) - indicate a slowness of turning even in the prodromal phases of PD and even in absence subthreshold parkinsonism.

In TUG performed at fast speed, the differences of turning duration, and angular velocities between controls and PD were more evident compared to controls. Conversely, none of these parameters differentiated iRBD from controls – this is explained by a significantly higher percentage of variation change of duration between normal and fast speed in iRBD, compared to that of PD and of controls.

We hypothesize that the lack of differences in the high-speed TUG between iRBD and controls could be due to a greater involvement of cortical circuits, which are proved to be hyperactivated in iRBDs during motor tasks (18). Moreover, we hypothesize that subcortical networks responsible for usual mobility performance may be affected by the neurodegenerative process at an earlier time point compared with cortical networks responsible for challenging mobility performances in older adults (19, 20). When parkinsonism is present, conversely- the differences between patients and controls appeared to be even wider in challenging conditions compared to normal speed. This fits with several studies addressing gait and mobility changes in early PD (21).

Inertial sensors are therefore promising tools that are able to detect subtle changes that are not captured by the evaluation of the time required to complete the task.

The study entails some limitations. First, although the sample size is large for these specific groups, it may still be too small to consider all confounding factors. However, the pattern of non-motor features in our iRBD cohort is comparable to previous cohorts with confirmed high conversion rates(22), which argues for the relevance of the reported findings. An important strength of the study is the strict inclusion criteria for iRBD (PSG-confirmed, absence of subthreshold parkinsonism) and PD (drug-naïve and confirmed by dopaminergic imaging).

Second, the study design did not allow the calculation of the predictive value of digital parameters to conversion and need to be verified in on-going longitudinal multi-centre studies. Third, we were not able to include an unsupervised assessment, as the features related to bradykinesia are expected to be more evident in the home-setting compared to supervised evaluation in the clinic (23). Still, the data showed an important difference of turning time and speed even in absence of changes in total TUG time in iRBD, thus confirming the potential usability of this measure as progression marker. One of most important current limitation in prodromal PD research is indeed the lack of markers able to track subtle changes of disease progression at this stage. This issue is

highly relevant for the research community, considering the upcoming availability of disease-modifying treatments.

In summary, this Mobile health technology-based study shows altered turning behavior in iRBD compared to controls, even in absence of subthreshold parkinsonism and with a similar total TUG duration. Longitudinal studies are warranted to establish the sensitivity to change of turning alterations over time in prodromal PD phases. Understanding the underlying mechanisms of these mobility deficits could be pivotal to develop outcome parameters for pharmacological and non-pharmacological strategies in this early phase of alpha-synucleinopathies.

METHODS

Participants and clinical assessment

The prospective cross-sectional study enrolled a total of 148 participants at the outpatient Movement Disorder clinic, Neurology Unit at the University of Brescia, Italy from April 2018 to November 2022 and at the Sleep disorder Centres of ASST Spedali Civili of Brescia and San Raffaele University, Milan. The research protocol was approved by the Ethics Committee of the Brescia Hospital, Brescia, Italy (DMA study, NP 1471). Written informed consent was obtained from all participants.

The study involved established iRBD individuals with a PSG-proven diagnosis and Drug-naïve PD patients (4).

Established iRBD individuals were included based on the following criteria: i) history of dream-enacting behaviour; ii) PSG proven REM sleep with sustained electromyographic (EMG) activity; (iii) absence of known pathological neurologic condition; iv) lack of motor or cognitive complaints, v) normal magnetic Resonance imaging (MRI) and vi) lack of another sleep disorder, medical disorder or medication interfering with gait, or substance abuse (2); vii)-lack of abnormalities of gait at standard neurological examination.

Drug naïve patients with clinically evident PD symptoms (4) were included. The following exclusion criteria were applied: i) symptoms or features suggesting atypical parkinsonism (Postuma et al. 2015); ii) dementia (4, 5); iii) other neurological disorders or medical conditions potentially associated with gait alterations; iv) abnormal MRI v) need of walking aids; vi) normal nigrostriatal dopaminergic imaging; vii) bipolar disorder, schizophrenia, history of drug or alcohol abuse or impulse control disorder.

Controls were recruited from patients' families and from healthy volunteers.

Each participant underwent a standard medical and neurological examination, including the Movement Disorder Society- Unified Parkinson Disease Rating Scale (MDS-UPDRS) (6), the Non-Motor Symptoms Scale for Parkinson's Disease (NMSS)(7), the Sniffing Sticks for the assessment of olfaction (8), the Montreal Cognitive Assessment (MoCA) for the assessment of cognitive function (9).

Mobile health technology-instrumented Timed Up and Go assessment

All individuals were asked to perform the Timed Up and Go test (TUG). The TUG test was chosen because it embeds turning in a sequence of everyday movements, providing a more realistic patient's ability to turn. The test was performed in clockwise and counterclockwise direction at normal and fast speed according to previous protocols (10, 11).

During the assessment, Rehagait® Inertial Measurement Unit with gyroscopes and accelerometers (Hasomed, Magdeburg, Germany) were used. The device was placed at the level of the fifth lumbar spine segment close to the centre of mass. Signal raw data were downloaded to a computer, segmented into individual walking trials using time stamps, and analysed by a bespoke MATLAB (R2021a) program. For the detection of turns and the extraction of turn parameters, we used a previously published algorithm developed by Pham et Al (13). The development of the algorithm for turning detection and analysis was performed with MATLAB R2015b and consisted of three steps:

6DOF attitude estimation, turning detection, and turning analysis. The basic principle of the 6DOF attitude estimation is built on the relative orientation of the sensor with respect to the reference frame (global frame, G-frame), using a rotation matrix GSR (S stands for sensor frame, S-frame). In a final step of the 6DOF attitude estimation, GSR was converted to Euler angles (roll–pitch–yaw) for the detection of turning, where roll represents the angle displacements around X, pitch around Y, and yaw around Z. Only yaw (i.e., the angular displacement around the Z axis) was considered for the next step.

Angular displacement around the Z axis was then plotted, where the start of a turn to the right was defined by a change from an increase to a decrease and the end by the change from a decrease to an increase. For the definition of a turn to the left, the situation was defined vice versa. The duration (horizontal component of the line) and magnitude (vertical component of the line) of each turn were determined between the start to end of the turn.

Turn direction was identified by integration of raw data from the gyroscope. A negative integration value was defined as a left turn and a positive integration as a right turn.

The turn metrics were duration, mean angular velocity and peak angular velocity (in degrees per second) of every turn (13).

Only variables with a percentage of missing data lower than 5% were considered. Outliers were defined by a value higher or lower than 3 standard deviation of the disease-specific group and were excluded from the analyses.

The percentage of variation in fast speed in comparison to normal speed was calculated as follow for mean and peak angular velocities: $\% \text{ variation} = \frac{\text{fast velocity} - \text{normal velocity}}{\text{normal velocity}}$.

Statistical analyses.

Differences in demographic and clinical parameters between participants with iRBD, PD and controls were checked for their normality distribution with Shapiro-Wilk test and assessed with

parametric ANOVA for continuous variables and chi-square test for categorical variables.

Accordingly, either parametric or non-parametric univariate analysis were used for between-group analyses, adjusting for age and sex. Similar to Van Uem et Al (10), mean values from the clockwise and counterclockwise turns were used. Between-group differences from the turning parameters were calculated using ANCOVA adjusting for age and sex. Bonferroni correction was applied to the post-hoc pairwise comparisons.

All analyses were 2-tailed, and $p < 0.05$ considered as statistically significant. For the analyses of digital parameters, a multiple comparison adjustment was adopted and statistical significance set to $p < 0.006$. Statistical analyses were performed using IBM SPSS statistics version 26.

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Table 1. Demographics and clinical characteristics in controls, iRBD and PD

	CP	iRBD	PD	P-value*
Participants [n]	65	23	60	
Age [y]	69±6	72±6	68±8	0.055 ^a
Height [m]	1.7±0.1	1.7±0.1	1.7±0.1	0.340 ^a
Sex M/F (%M)	26/39 (40)	19/4 (83)	32/28 (53)	0.002 ^b *#
Disease Duration [y]	-	-	1±1	
RBD symptoms duration		4±3		
MoCA (0-30) [pts]	27±2	25±3	24±3	<0.001 ^c §
MDS-UPDRS-I (0-52) [pts]	1±12	6±5	5±5	<0.001 ^c *§
MDS-UPDRS-II (0-52) [pts]	0±1	1±1	4±4	<0.001 ^c §#
MDS-UPDRS-III (0-132) [pts]	2±3	2±2	16±10	<0.001 ^c §#
RBDSQ (0-13) [pts]	0±1	9±3	3±3	<0.001 ^c *#§
NMSS (0-360) [pts]	1±1	22±17	21±21	<0.001 ^c *§
Sniffing Sticks (0-12) [pts]	9±2	5±3	5±2	<0.001 ^c *§
Hyposmic subjects [n] (%)	4 (6%)	14 (61%)	45 (75%)	<0.001 ^c *§

a. Parametric ANOVA

b. Chi Square test

c. Non-parametric comparison corrected for age and sex

Pairwise with Bonferroni Correction for 3 comparisons (* iRBD vs CP; § PD vs CP; # iRBD vs PD). *p*-value <0.05 was considered as statistically significant

Abbreviations: BDI, Beck Depression Inventory; CP, control participants; iRBD, subjects with idiopathic REM sleep behavior disorders; MDS-UPDRS, Movement Disorder Society Unified Parkinson's disease Rating scale; MoCA, Montreal Cognitive Assessment; NPI, neuropsychiatric inventory; NMSS, non motor symptom scale; PD, Parkinson's disease patients; RBDSQ, RBD screening questionnaire.

Table 2. TUG parameters in controls, iRBD and PD

	CP	iRBD	PD	P-value*	iRBD-CP difference	PD-CP difference	PD-iRBD difference
TUG NORMAL SPEED							
TUG duration (s)	11.3±2.09	12.8±1.8	14.0±3.5	<0.001 ^b §	1.4±0.8	2.4±0.5	1±0.8
Duration Of Turns (s)	2.3±0.4	2.8±0.4	3.0±0.6	<0.001 ^a §	0.5±0.1	0.7±0.1	0.2±0.1
Mean Angular Velocity	82±14	67±8	61±15	<0.001 ^a *§	-15.0±3.4	-20.4±2.4	-5.4±3
Peak Angular Velocity	194±34	152±22	138±28	<0.001 ^a *§	-41.9±7.7	-54.0±5.5	-12.1±7.5
TUG FAST SPEED							
TUG duration (s)	8.9±1.8	9.2±0.9	10.9±2.4	<0.001 ^b §#	0.3±0.6	1.8±0.4	1.5±0.5
% Variation TUG duration	-21.8±8.8	-27.7±6.4	-20.3±6.7	0.011 ^b *#			
Duration Of Turns (s)	1.9±0.3	2.2±0.4	2.5±0.5	<0.001 ^b §#	0.3±0.1	0.6±0.1	0.3±0.1
% Variation Turning	-16.6±12.6	-21.22±12.3	-13.8±10.2	0.042 ^a			
Mean Angular Velocity	98±18	85±15	72±16	<0.001 ^a §#	-12.9±4.2	-24.4±2.9	-11.5±4.1
%VariationMeanVelocity	20.9±16.4	26.1±18.4	16.8±13.2	0.219 ^b			
Peak Angular Velocity	235±40	203±37	172±35	<0.001 ^a §#	-32±9.4	-62.8±6.7	-30.8±9.2
%VariationPeakVelocity	22.6±13.2	30.9±20.3	22.9±14.8	0.822 ^b			

a. Parametric comparison corrected for age and sex

b. Non-parametric comparison corrected for age and sex

Pairwise with Bonferroni Correction for 3 comparisons (* iRBD vs CP; § PD vs CP; # iRBD vs PD). p-value <0.006 was considered as statistically significant for digital extracted variables.

Abbreviations: CP, control participants; iRBD, subjects with idiopathic REM sleep behavior disorders; PD, Parkinson's disease patients; TUG, timed up and go test.

C) Counting Steps Instead of Sheep: Gait in naïve Parkinson's With and Without Sleepiness

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In progress

INTRODUCTION

Gait disturbances are hallmark symptoms of Parkinson's disease (PD), significantly contributing to impaired mobility and reduced quality of life. These disturbances encompass a spectrum of motor impairments, including reduced step length, increased stride variability, and diminished gait speed. While the motor symptoms of PD have been extensively studied, emerging evidence highlights the role of non-motor symptoms, such as sleep disturbances, in exacerbating these impairments. Sleep disturbances are prevalent in up to 75–90% of individuals with PD, including insomnia, REM sleep behavior disorder (RBD), and fragmented sleep patterns, all of which can contribute to heightened daytime fatigue, diminished physical performance, and altered motor control. [1]

Quality of sleep is intricately linked to motor function through several neurophysiological mechanisms. Sleep plays a critical role in motor learning, muscle recovery, and neuroplasticity. In individuals with neurodegenerative disorders, impaired sleep may exacerbate motor deficits by disrupting these processes, alongside contributing to cognitive decline, which is often co-morbid with gait abnormalities. Specifically, disrupted sleep in PD has been associated with increased postural instability and impaired reaction times, suggesting a compounding effect on gait abnormalities. [2]

While the interplay between sleep quality and gait has been increasingly studied in the general elderly population, there is limited but growing literature investigating this relationship in neurodegenerative conditions like PD. Research in other neurodegenerative conditions, such as Alzheimer's disease, has also suggested that poor sleep exacerbates motor and cognitive impairments, further underscoring the significance of addressing sleep quality as a modifiable factor in managing disease symptoms. [3]

However, few studies have directly compared gait parameters between PD patients with and without sleep disturbances, leaving a gap in understanding the specific impact of sleep quality on motor symptoms in this population.

In this study, we aim to address this gap by comparing gait parameters between PD patients with and without sleep disturbances. By delineating the influence of sleep quality on gait, our findings may provide insights into the broader implications of sleep management in PD and other neurodegenerative diseases.

METHODS

Participants and clinical assessment

The prospective cross-sectional study enrolled a total of 61 participants at the outpatient Movement Disorder clinic, Neurology Unit at the University of Brescia, Italy from April 2018 to November 2022 and at the Sleep disorder Centres of ASST Spedali Civili of Brescia. The research protocol was approved by the Ethics Committee of the Brescia Hospital, Brescia, Italy (DMA study, NP 1471). Written informed consent was obtained from all participants.

The study involved established Drug-naïve PD patients with clinically evident PD symptoms.

The following exclusion criteria were applied: i) symptoms or features suggesting atypical parkinsonism [4]; ii) dementia [4, 5]; iii) other neurological disorders or medical conditions potentially associated with gait alterations; iv) abnormal MRI v) need of walking aids; vi) normal

nigrostriatal dopaminergic imaging; vii) bipolar disorder, schizophrenia, history of drug or alcohol abuse or impulse control disorder.

Each patient underwent a standard medical and neurological examination, including the Movement Disorder Society- Unified Parkinson Disease Rating Scale (MDS-UPDRS) [6], the Non-Motor Symptoms Scale for Parkinson's Disease (NMSS)[7], the Sniffing Sticks for the assessment of olfaction [8], the Montreal Cognitive Assessment (MoCA) for the assessment of cognitive function [9].

Sleepiness was assessed using Epworth Sleepiness Scale (ESS) [10]. Fourteen patients scored ≥ 7 on the ESS and were considered as the PD group with sleepiness (PDSleep). Another forty-seven PD patients without sleepiness (ESS < 7 , PDAwake), matched for age, sex, cognitive function, and disease severity, were also included in the study.

Gait assessment with mobile health technologies and data extraction

Sixty-seven patients underwent gait assessment under supervised conditions using five OPAL[®] sensors device placed one on the lower back, two at the wrist and two at the dorsal part of the feet. Participants walked a 20-meter walkway at self-selected speed, at fast speed and in dual tasking conditions with motor task and cognitive task while walking [11].

Data processing and extracted parameters

Raw data were processed using Matlab R2022b (MathWorks, Nantick, MA, USA). To analyze gait parameters, raw data from the were used for assessments. To ensure no systematic biases between assessments in terms of heterogeneity, usage, and accuracy, raw accelerometer and gyroscope data were processed using validated algorithm [12].

As described in reference [12], the raw accelerometer and gyroscope data were processed to detect gait events.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 26. Comparisons of clinical and demographic parameters between PD-Sleep and PD-Alert were performed using the Mann-Whitney U test and Fisher's exact test. Step time, step time variability, and step time asymmetry and step length [29] were then compared between groups using the Mann-Whitney non-parametric analysis, adjusting for age, sex, height, and disease duration. Correlations between ESS values and gait parameters were assessed using partial correlation analyses, adjusting for age, sex, height and onset of disease. A p-value < 0.05 was considered statistically significant.

RESULTS

The PD-Sleep group did not significantly differ from the PD-Alert group in term of age, disease duration, gender distribution, and MDS-UPDRS-III, MoCA, and BMI scores, respectively. (Table 1) Step length and step time significantly differ between PD-Sleep and PD-Alert in all gait conditions ($p < 0.05$) except step time in fast pace. Variability and asymmetry didn't show any difference in all conditions. Moreover, gait parameters from the entire cohort did not significantly correlate with any demographic or clinical parameter.

Details are provided in Table 2. No correlation between digital parameters and ESS scores were found in the whole group. In PD-Sleep, ESS total score correlated with the step length in dual tasking conditions, both motor and cognitive task (respectively: $r = 0.46$, $p = 0.042$; $r = 0.56$, $p = 0.013$).

DISCUSSION

The findings of this study provide valuable insights into the relationship between sleep disturbances and gait parameters in individuals with Parkinson's disease. By comparing drug-naïve PD patients with (PD-Sleep) and without (PD-Alert) sleepiness, this study showed the nuanced impact of sleep disturbances on motor function, particularly gait, in this population.

The significant differences in step length and step time between PD-Sleep and PD-Alert across various gait conditions (except step time at a fast pace) suggest that sleep disturbances contribute to specific aspects of gait impairment in PD. Notably, the lack of differences in variability and asymmetry indicates that sleep disturbances may primarily influence gait parameters linked to step efficiency rather than rhythm or stability. This finding aligns with prior research emphasizing the role of sleep in muscle recovery and neuroplasticity, processes crucial for efficient gait patterns.

[13, 14]

The observed correlation between Epworth Sleepiness Scale (ESS) scores and step length during dual-task conditions in the PD-Sleep group further supports the hypothesis that sleep disturbances exacerbate motor deficits in cognitively demanding scenarios. Dual-task conditions require simultaneous processing of motor and cognitive inputs, and disrupted sleep may compromise the brain's capacity to allocate resources effectively, leading to shorter steps. [15]

This aligns with the understanding that fragmented sleep can impair executive function and motor control, particularly in challenging contexts [1].

The findings underscore the importance of assessing and addressing sleep disturbances in PD patients as part of a holistic approach to managing motor symptoms. Interventions targeting sleep quality—such as cognitive-behavioral therapy for insomnia (CBT-I), pharmacological treatments for sleep disorders, or lifestyle modifications—could potentially mitigate gait impairments [16].

Additionally, these results highlight the utility of mobile health technologies for detailed gait

assessment, offering a non-invasive method to monitor disease progression and response to therapy [17].

Despite its strengths, including the use of drug-naïve patients and rigorous statistical adjustments, the study has several limitations. The relatively small sample size, particularly in the PD-Sleep group, may limit the generalizability of the findings. Furthermore, the cross-sectional design precludes causal inferences; longitudinal studies are needed to determine whether improving sleep quality can reverse or slow gait deterioration in PD. Future research should also explore the mechanisms underlying the observed associations, such as alterations in specific brain networks or neurotransmitter systems affected by sleep disturbances. [18]

This study bridges an important gap in understanding the interplay between sleep disturbances and gait abnormalities in PD. By demonstrating that sleep quality significantly influences gait parameters, particularly under dual-task conditions, these findings advocate for incorporating sleep management into PD care strategies. Addressing sleep disturbances may not only improve overall quality of life but also enhance mobility and reduce the risk of falls in this vulnerable population.

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Table 1. Clinical characteristics of the patients

	PD-Sleep	PD-Alert	p-value
Participants [n]	14	47	
Age [years]	70.9±8.8	68.9±6.6	0.263 ^a
Sex [% male]	40.1	42.4	0.976 ^b
Disease duration [years]	1.5±1.1	1.6±0.8	0.430 ^a
MDS-UPDRS-III	21.6±10.5	19.8±10.3	0.438 ^a
NMSS	39.6±28.0	29.6±28.5	0.164 ^b
BDI	7.9±6.3	7.2±5.7	0.635 ^b
Sniffing Test	5.4±2.5	5.5±1.9	0.703 ^b
Height [m]	1.67±0.08	1.68±0.10	0.505 ^a
MoCA Score (0-30)	22.4±5.6	24.1±4.5	0.106 ^a
ESS score	10.1±1.8	3.6±2.1	<0.001^a

^a Mann-Whitney-U test, ^b Fisher's exact test.

BMI, body mass index; NMSS Non-Motor Symptoms Scale; BDI Beck Depression Index; MoCA,

Montreal Cognitive Assessment; ESS, Epworth Sleepiness Scale.

Table 2. Clinical characteristics of the patients

GAIT PARAMETERS	PD-Sleep	PD-Alert	p-value
Self Selected Speed			
Step Time [s]	0.56±0.04	0.52±0.04	0.044
Step Time Variability [s]	0.07±0.07	0.07±0.04	0.447
Asymmetry Index	0.03±0.02	0.02±0.02	0.213
Step Lenght [cm]	0.52±0.05	0.56±0.05	0.013
Fast Speed			
Step Time [s]	0.48±0.05	0.48±0.05	0.994
Step Time Variability [s]	0.02±10.8	0.02±0.02	0.929
Asymmetry Index	0.08±0.07	0.07±0.05	0.413
Step Lenght [cm]	0.56±0.06	0.60±0.07	0.044
Checking Boxes			
Step Time [s]	0.56±0.06	0.52±0.05	0.039
Step Time Variability [s]	0.08±0.04	0.08±0.06	0.574
Asymmetry Index	0.03±0.02	0.02±0.01	0.561
Step Lenght [cm]	0.51±0.06	0.55±0.06	0.045
Serail Subtractions			
Step Time [s]	0.55±0.06	0.51±0.05	0.030
Step Time Variability [s]	0.08±0.07	0.06±0.05	0.605
Asymmetry Index	0.03±0.02	0.02±0.01	0.407
Step Lenght [cm]	0.51±0.08	0.56±0.07	0.035

Comparison between PD-Sleep and PD-Alert have been performed using non-parametric multivariate analyses adjusted for the effect of age, sex, height, disease duration and number of steps.

2.2 FATIGUE

A) Unsupervised but not supervised gait parameters are related to fatigue in parkinson's disease: a pilot study.

Pilotto A, **Rizzardi A**, Zatti C, Hansen C, Donini A, Romijnders R, Maetzler W, Padovani A.

Unsupervised but not supervised gait parameters are related to fatigue in Parkinson's disease: a pilot study. *Front Aging Neurosci.* 2023 Nov 21;15:1279722. doi: 10.3389/fnagi.2023.1279722.

PMID: 38076532; PMCID: PMC10702762.

INTRODUCTION

Fatigue is a common and disabling symptom in Parkinson's disease (PD) [1-3] affecting between 30 and 70 percent of PD patients [4]. This makes fatigue an important target for pharmacological and non-pharmacological interventions [5, 6]. The dramatic impact fatigue on quality of life [7] may be mediated by various PD-related symptoms, with movement and mobility deficits playing a central role. For example, it was shown that, in PD, fatigue is associated with the Postural Instability/Gait Difficulty (PIGD) subtype [8, 9] and higher total motor scores [8, 9]. In the general population, fatigue can have a significant impact on mobility and particularly on gait parameters, namely a decrease of walking speed and step length and an increase of step variability [10-11]. In PD, however, the relationship between fatigue and gait-related parameters has never been explored using digital assessment and thus represent a still debated issue in clinical research [12].

Mobile health technologies (MHT) enable the collection of quantitative and qualitative aspects of gait with high accuracy both in the professional clinical-scientific environment (supervised condition, SC) and in the home of users or patients (unsupervised condition, UC). Several studies suggest that assessment under SC, but not under UC, underestimate PD-related gait deficits [13, 14]. This might especially true for fluctuating symptoms particularly sensitive to motivation and alertness such as fatigue [1-3,12]

We therefore investigated in this pilot prospective observational study gait changes in PD patients with and without fatigue matched for age, sex, motor and cognitive severity. Aim of the study was to evaluate the relationship between gait parameters and fatigue using MHT, with the hypothesis that an home unsupervised assessment might be more informative compared to supervised clinical evaluation in showing this association.

METHODS

Participants and clinical assessment

A total of 80 patients with a clinically established PD [15] with at least one year of follow-up after diagnosis and beneficial response to dopaminergic treatment from the outpatient Movement disorder Clinic, Neurology Unit at the University of Brescia, Italy from March 2018 to December 2022. The research protocol was approved by the Ethics Committee of the Brescia Hospital, Brescia, Italy (DMA study, NP 1471/22). Written informed consent was obtained from all participants.

Only patients under stable dopaminergic treatment [15] were included. Exclusion criteria were features suggesting atypical parkinsonism [15], a diagnosis of dementia [16], impulse control disorder, symptomatic orthostatic hypotension, motor fluctuations (UPDRS-IV ≥ 1), other neurologic disorders or medical conditions potentially affecting gait, need of walking aids, major depressive and bipolar disorder needing pharmacological treatment, schizophrenia, and history of drug and alcohol abuse.

All patients underwent a neurological examination, including the Movement Disorder Society- Unified Parkinson Disease Rating Scale (MDS-UPDRS) part III and IV [17], the Hoehn and Yahr (H&Y) staging [18] and a global cognitive screening using the Montreal Cognitive Assessment test (MOCA) [19]. Fatigue was assessed using the PD Fatigue Scale (PFS-16) [20]. Twenty-one patients had ≥ 8 points on the PFS-16 and were considered as the PD group with fatigue (PD-F) [5]. Twenty-one PD

patients without fatigue (PFS16 <8, PD-N) matched for age, sex, cognitive function and disease severity were also included.

Gait assessment with mobile health technologies and data extraction

This selected 42 patients underwent gait assessment under SC and UC (Supplementary figure 1).

Gait assessment under SC was performed with a RehaGait® device on the lower back (Hasomed GmbH, Magdeburg, Germany) [21]. Participants walked a 20m walkway up and down first with self-selected, then with fast speed [22]. Gait assessment under UC was performed with a Move IV® device on the lower back (Movisens GmbH, Karlsruhe, Germany) over four consecutive days [23]. Patients were randomly assigned to start the evaluation with either supervised or unsupervised assessment.

Inertial sensors used in the study for supervised and unsupervised data evaluate the same gait parameters (all included in the analyses) based on lower back algorithms, as extensively described in the ComON study design and in the next session [21]

Data Processing and Extracted Parameters

Raw data were processed using Matlab R2022b (MathWorks, Nantick, MA, USA). To analyze gait parameters the raw data of the IMU from the lower back was used for both supervised and unsupervised assessments. To ensure no systematic biases between assessments in terms of heterogeneity, usage and accuracy, raw data of accelerometer and gyroscope from the two assessments were processed with the same validated algorithm [24, 25].

As described in the reference [25], the raw accelerometer and gyroscope data were processed to first detect the gait events. From the home assessment mean gait parameters per walking bout (WB) was calculated. WBs were determined with more than 3 consecutive steps and then were

considered for further analysis. UC gait bouts were split into short (4-19 steps) and long gait bouts (≥ 20 steps) based on the number of steps [26-28] for each day of recording.

Statistical analysis

Statistical analyses were performed using IBM SPSS statistics version 26. Comparisons of clinical and demographic parameters between PD-F and PD-N were done with Mann-Whitney-U and Fisher's exact test. Step time, step time variability and step time asymmetry [29] were then compared between groups in SC and UC, using Mann Whitney non-parametric analysis and adjusting for age, sex, height and disease duration. Correlations between PFS-16 values and gait parameters were done using partial correlation analyses and again adjusting for age, sex, height and disease duration. $P < 0.05$ was considered statistically significant.

RESULTS

The PD-F group did not significantly differ from the PD-N group in term of age, disease duration, gender distribution, and MDS-UPDRS-III, MoCA, and BMI scores, respectively (Table 1). Under SC, none of the investigated gait parameters did significantly differ between PD-F and PD-N, neither during self-selected, nor during fast pace. Moreover, gait parameters from the SC of the entire PD cohort did not significantly correlate with any demographic or clinical parameter.

Under UC, PD-F showed higher step time ($p=0.02$), step time variability ($p=0.005$) and step time asymmetry ($p=0.01$), compared to PD-N. These differences were more pronounced in the long gait bouts, and less pronounced in the short gait bouts. Details are provided in Table 2. No correlation between digital parameters and PFS-16 scores were found in the whole group. In PD-F, PFS-16 total score correlated with the step time in the longer ($r=0.46$, $p=0.05$) and shorter bouts ($r=0.56$, $p=0.013$), whereas no correlation was observed in SC.

DISCUSSION

This pilot study addressed the complex relationship between fatigue and gait changes in PD patients under supervised and unsupervised home assessment. The findings showed, to our best knowledge for the first time, that subjects with higher fatigue levels present worse gait parameters compared with subjects not reporting fatigue but only when measured in UC using MHT. Conversely, gait parameters as collected in the clinic did not reveal any significant difference between PD patients with and without fatigue. These novel results are of high interest for the research community, as the association between fatigue and “measurable” mobility parameter is still a debated issue in research.

Compared to PD-N, PD-F indeed exhibited in the home environment longer step time and increased step time variability and asymmetry index, which are the typical PD-associated features [33]. Longer step time and step time asymmetry indicate more severe gait alterations in PD-F, whereas increased gait variability has been consistently associated with an increased risk of accelerated progression and an increased risk of falls in PD [34].

The study design allowed a matching of PD with similar disease duration and severity, dopaminergic treatment and cognitive function excluding patients with depression or motor fluctuations, in order to minimize the variables potentially influencing both fatigue and motor performances at home and in the clinic [4, 6, 13, 30-32]. The differences observed between supervised and unsupervised environment are probably due to different factors, including alertness, motivation, the white-coat and the reverse white-coat effects (respectively worsening and improvement of a clinical parameter measured in clinical setting) and the Hawthorne effect (i.e. change in participants' behavior due to the awareness that they are being studied) [14]. In fact, these factors may mask fatigue aspect and

argue for an easier and more consistent role for unsupervised home evaluation for such type of target-variable and assessments.

Of interest, the differences observed in step time and step time variability between the groups in UC were, at least upon visual inspection, more prominent in long walking bouts that are more comparable to supervised analyses [24]. Longer bouts may also reflect a more automatic walking conditions with less alertness and thus higher risk of both fatigue and classical dopamine-related features.

Several further study limitations should be acknowledged. First, the sample size and the pilot nature of this study did not allow specific sex- or fatigue-subcategory analyses (i.e mental vs physical), that need to be verified in larger studies. Second, the cross-sectional study design cannot evaluate changes in fatigue and mobility over time. Third, this study excluded patients with major depression, but the link between subtle depressive symptoms psychosocial well-being and fatigue should be definitively further explored in larger samples. Fourth, the study excluded a priori PD patients with motor fluctuations to limit the variability of both fatigue and mobility. Fatigue, however, is known to represent an important symptom of L-dopa-induced fluctuation and further studies focused on this specific population are pivotal also to challenge new pharmacological approaches.

Limitations notwithstanding, this study shows that fatigue in PD is associated with specific gait changes which are detectable in home-based environment using MHT technology.

These novel findings can be further investigated in ongoing studies, such as the large IMI consortium IDEA-FAST (<https://idea-fast.eu>) including home-based diaries and longer unsupervised evaluation, which will enable a deeper understanding of the complex relationship between fatigue, mobility and daily activities.

Data Availability

The data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Table 1. *Clinical characteristics of the patients*

	PD-N	PD-F	p-value
Participants [n]	21	21	
Age [years]	64.9±8.0	69.6±7.5	0.061 ^a
Sex [% male]	71.4	52.4	0.204 ^b
Disease duration [years]	5.5±3.1	4.6±2.8	0.230 ^a
MDS-UPDRS-III (0-132)	12.8±5.6	15.9±9.5	0.378 ^a
MDS UPDRS-III gait subscore [% ≥1 score]	38.1	42.9	0.753 ^b
MDS UPDRS-III tremor subscore [% ≥1 score]	33.3	47.6	0.217 ^b
MDS UPDRS-III rigidity subscore [% ≥1 score]	71.4	66.6	0.683 ^b
Height [m]	1.70±0.10	1.67±0.08	0.287 ^a
MoCA Score (0-30)	27.4±2.1	26.9±2.1	0.646 ^a
LEDD	370±209	345±212	0.693 ^a
L-dopa LEDD	275±181	305±189	0.533 ^a
DA-LEDD	53.4±62.0	47.1±55.5	0.725 ^a
PFS-16 Score (0-16)	2.1±2.2	9.3±2.1	<0.001^a

^a Mann-Whitney-U test, ^b Fisher`s exact test. BMI, body mass index; DA-LEDD, dopamine-agonist

LEDD; MoCA, Montreal Cognitive Assessment; L-dopa-LEDD, Levodopa LEDD; LEDD, levodopa

equivalent daily dose; PFS-16, Parkinson`s disease fatigue score.

Table 2. Differences in gait parameters between PD with and without fatigue in supervised and unsupervised conditions. Values have been indicated as mean (25th-75th percentile).

GAIT PARAMETERS	PD-N	PD-F	p-value ^c
Supervised assessment			
Straight Walk Self-Selected Pace			
Steps [N]	113 (88-131)	108 (96-119)	0.273 ^c
Step time [s]	0.53 (0.45-0.67)	0.55 (0.49-0.70)	0.116 ^c
Step Time variability[s]	0.04 (0.02-0.10)	0.05 (0.03-0.12)	0.264 ^c
Step time asymmetry	0.02 (0.00-0.04)	0.03 (0.00-0.08)	0.125 ^c
Straight Walk Fast Pace			
Steps [N]	118 (102-159)	123 (110-159)	0.320 ^c
Step time [s]	0.46 (0.38-0.57)	0.49 (0.38-0.54)	0.108 ^c
Step Time variability[s]	0.04 (0.02-0.09)	0.06 (0.02-0.09)	0.140 ^c
Step time asymmetry	0.01 (0.00-0.04)	0.02 (0.00-0.05)	0.207 ^c
Unsupervised assessment			
Daily Bout			
Steps [N]	31 (11.1-83.5)	30 (14.7-74.3)	0.125 ^d
Step Time [s]	0.74 (0.66-0.78)	0.78 (0.69-0.90)	0.007^d
Step Time Variability[s]	0.24 (0.17-0.39)	0.29 (0.23-0.37)	0.004^d
Step time asymmetry	0.11 (0.07-0.21)	0.13 (0.09-0.20)	0.004^d
Gait bouts < 20 steps			
Steps [N]	14 (8.5-16.2)	15 (8.0-16.0)	0.269 ^d
Step Time [s]	0.78 (0.72-0.87)	0.83 (0.70±0.99)	0.017^d
Step Time Variability [s]	0.31 (0.18-0.40)	0.34 (0.28-0.43)	0.017^d
Step time asymmetry	0.16 (0.09-0.23)	0.17 (0.11-0.24)	0.032^d
Gait bouts ≥20 steps			
Steps [N]	52 (32-167)	50 (30-153)	0.250 ^d
Step time [s]	0.67 (0.61-0.73)	0.69 (0.65-0.80)	0.013^d
Step Time variability [s]	0.19 (0.15-0.34)	0.23 (0.18-0.30)	0.002^d
Step time asymmetry	0.06 (0.04-0.08)	0.07 (0.05-0.09)	0.010^d
Longest bout			
Steps [N]	892 (58-3089)	500 (56-2476)	0.175 ^d
Step time [s]	0.55 (0.46-0.69)	0.59 (0.53-0.88)	0.009^d
Step Time variability [s]	0.05 (0.02-0.21)	0.08 (0.05-0.36)	0.029^d
Step time asymmetry	0.01 (0.00-0.03)	0.02 (0.00-0.06)	0.005^d

^c Comparison between PD-N and PD-F have been performed using non-parametric multivariate analyses adjusted for the effect of age, sex, height, disease duration and number of steps.

^d Comparison between PD-N and PD-F have been performed using non-parametric multivariate analyses adjusted for the effect of age, sex, height, disease duration and number of steps.

2.3 ACTIVITIES OF DAILY LIVING

A) Motor reserve impact on turning in de novo parkinson's disease patients

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INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor symptoms such as bradykinesia, rigidity, tremor, and postural instability. Alterations of turning are key in the early phases of Parkinson disease [1]. Turning while walking involves complex motor coordination, balance, and agility. Even before the full onset of motor symptoms like tremors and

rigidity, subtle changes in motor control, such as turning difficulty, can arise to the early degeneration of dopaminergic neurons in the basal ganglia, which plays a critical role in initiating and regulating movement. These early motor changes can be detectable as slight asymmetries, hesitation, or “freezing” episodes during turning, indicating the progressive motor impairment associated with PD [2]. Studies suggest that alterations in turning could help clinicians predict PD and monitor its progression (REF?). Objective turning tests using innovative technology namely wearable sensors, can track subtle changes that point to developing Parkinsonian patterns [3]. While the pathophysiology of Parkinson's disease has been extensively studied, there remains considerable variability in the clinical presentation and progression of severity and motor performance among individuals. This variability suggests that factors beyond the primary neuropathological changes contribute to the observed clinical outcomes.

One such factor is the concept of motor reserve (MR), an emerging area of interest in neurodegenerative research [4]. MR refers to the brain's ability to compensate for neural damage and maintain motor function despite pathological changes. This concept is akin to cognitive reserve [5], which has been extensively studied in Alzheimer's disease and other forms of dementia. Motor reserve may be influenced by various factors including genetics, lifestyle, physical activity, and overall brain health, which can modulate the impact of neuronal loss on motor function [6, 7]. Assessing motor reserve in the context of Parkinson's disease could aid in the prognostic evaluation of patients, allowing for more personalized treatment plans. This work aims to explore the concept of motor reserve in Parkinson's disease, examining its potential clinical implications. We seek to elucidate how motor reserve can impact on mobility in these patients to improve the management and quality of life of individuals living with Parkinson's disease.

METHODS

Participants and clinical assessment

The study was performed on sample from a single-center prospective observational cohort study.

The prospective cross-sectional study enrolled consecutive patients enrolled at the outpatient Movement Disorder clinic, Neurology Unit at the University of Brescia, Italy from January 2023 to March 2024. The research protocol was approved by the Ethics Committee of the Brescia Hospital, Brescia, Italy (DMA study, NP 1471, with its last version on April 22, 2022). Written informed consent was obtained from all participants.

Drug naïve patients with clinically evident PD symptoms [8] and healthy volunteers were included.

The following exclusion criteria were applied: i) symptoms or features suggesting atypical parkinsonism; ii) cognitive decline [8, 9]; iii) other neurological disorders or medical conditions potentially associated with gait alterations; iv) abnormal MRI v) need of walking aids; vi) normal dopamine uptake in nigrostriatal imaging.

Participants were also excluded if: <18 years old; presence or history of any psychiatric diseases (such as depressive, bipolar, or psychotic disorders), impulse control disorder, acute or non-compensated illnesses; clinical or drug-associated conditions causing cognitive or mood dysfunction;

Controls were recruited from healthy volunteers.

All patients underwent a neurological examination, including the Movement Disorder Society- Unified Parkinson Disease Rating Scale (MDS-UPDRS) [10], the Hoehn and Yahr (H&Y) staging [11] and a global cognitive screening using the Montreal Cognitive Assessment test (MOCA)[12]. Motor reserve was measured using the Motor Reserve Index questionnaire (MRIq) [13] and the median value was used to divide patients in two groups: high motor reserve (High MR) and low motor reserve (Low MR) patients.

Mobile health technology instrumented Timed Up and Go assessment and Gait Analysis

All individuals were asked to perform the Timed Up and Go test (TUG). The TUG test was chosen because it embeds turning in a sequence of everyday movements, providing a more realistic patient's ability to turn. The test was performed in clockwise and counterclockwise direction at normal and fast speed according to previous protocols [14, 15]. During the assessment, Rehagait® Inertial Measurement Unit with gyroscopes and accelerometers (Hasomed, Magdeburg, Germany) were used [16]. The device was placed at the level of the fifth lumbar spine segment close to the center of mass. Signal raw data were downloaded to a computer, segmented into individual walking trials using time stamps, and analyzed by a bespoke MATLAB (R2021a) program. For the detection of turns and the extraction of turn parameters, we used a previously published algorithm developed by Pham et al [17]. The development of the algorithm for turning detection and analysis was performed with MATLAB R2021a and consisted of three steps: 6DOF attitude estimation, turning detection, and turning analysis. The basic principle of the 6DOF attitude estimation is built on the relative orientation of the sensor with respect to the reference frame (global frame, G-frame), using a rotation matrix GSR (S stands for sensor frame, S-frame).

In the final step of the 6DOF attitude estimation, GSR was converted to Euler angles (roll–pitch–yaw) for the detection of turning, where roll represents the angle displacements around X, pitch around Y, and yaw around Z. Only yaw (i.e., the angular displacement around the Z axis) was considered for the next step. Angular displacement around the Z axis was then plotted, where the start of a turn to the right was defined by a change from an increase to a decrease and the end by the change from a decrease to an increase. For the definition of a turn to the left, the situation was defined vice versa. The duration (horizontal component of the line) and magnitude (vertical component of the line) of each turn were determined between the start to end of the turn. Turn direction was identified by

integration of raw data from the gyroscope. A negative integration value was defined as a left turn and a positive integration as a right turn. The turn metrics were duration, mean angular velocity and peak angular velocity (in degrees per second) of every turn [17]. Only variables with a percentage of missing data lower than 5% were considered. Outliers were defined by a value higher or lower than 3 standard deviations of the disease-specific group and were excluded from the analyses. The percentage of variation in fast speed in comparison to normal speed was calculated as follows for mean and peak angular velocities: $\% \text{ variation} = \frac{\text{fast velocity} - \text{normal velocity}}{\text{normal velocity}}$

Statistical analysis

Differences in demographic characteristics between patients with high and low MR were tested for normality distribution using Shapiro-Wilk test and assessed using parametric ANOVA for continuous variables and the chi-squared test for categorical variables.

The Shapiro Wilk test was used to test for normality in the assessment of gait parameters.

Accordingly, either parametric or non-parametric univariate analysis were used for between-group analyses, adjusting for age and sex. Similar to Van Uem et al [14] mean values from the clockwise and counterclockwise turns were used. Between-group differences from the turning parameters were calculated using ANCOVA adjusting for age and sex. For the analyses of digital parameters statistical significance was set to $p < 0.05$ uncorrected for multiple comparisons, for exploratory purposes. Statistical analyses were performed using IBM SPSS statistics version 26.

Due to the observational and exploratory nature of the study, no sample size calculation was performed.

RESULTS

Forty-two naïve PD patients and forty age-matched controls entered the study. All subjects completed all MHT procedure without discomfort.

The overall duration of TUG tests was longer in PD compared to controls, both at normal and fast speed. The HC subjects showed higher angular and peak angular velocity and lower duration of turns. ($p < 0.005$) (Supplementary Table 1)

Partial correlations adjusted for age and sex revealed a significant relationship between digital parameters both in self-selected and fast pace and MRIq. ($p < 0.05$). Subitems analysis revealed that section IV related to physical activities and section V related to care activities were strongly correlated with durations of turns, angular velocity and peak velocity (all $p < 0.01$) whereas section I and section III related to housework and leisure activities did not show any correlations. A negative but non-significant correlation was found between MRI and MDS-UPDRS-III. (Figure 1)

Participants were divided into two groups according to the median value of the MRIq: High motor reserve and low motor reserve patients.

High vs. low-MR PD were comparable for demographics but not in height, motor and cognitive severity and clinical variables. (Table 1).

High-MR PD showed lower duration of turns and higher angular velocity and peak angular velocity both at self-speed ($p < 0.005$) and at fast speed ($p < 0.05$). No differences were found in the rates of change between normal and fast conditions. (Table 3)

DISCUSSION

Our results showed that there was no significant difference in TUG test completion times between patients with high and low MR PD. However, even in the absence of substantial variations in motor severity, the digital evaluation of turning revealed significant disparities between these groups. Our

analyses detected significant subclinical differences below the minimal clinically significant TUG time duration of 3.5 seconds. The digital assessment focused on turning duration, mean and peak angular velocity, standard parameters proven to distinguish PD patients in various cohorts [18, 19]. Turning performances were evaluated in TUG tests conducted at normal and fast speeds, with normal speed tasks potentially being more sensitive. At normal speed, differences in turning duration and angular velocities between high and low MR patients were more pronounced. However, at fast speed, these parameters were less effective in differentiating the groups, likely due to a greater variation in duration changes between normal and fast speeds in low MR PD patients compared to high MR patients.

We hypothesize that this could be due to the activation of cortical circuits in low MR patients during motor tasks, compensating for early subcortical impairments. In contrast, subcortical networks responsible for routine mobility may be affected later in high MR patients, while cortical networks are more engaged in handling complex or demanding tasks. [20]

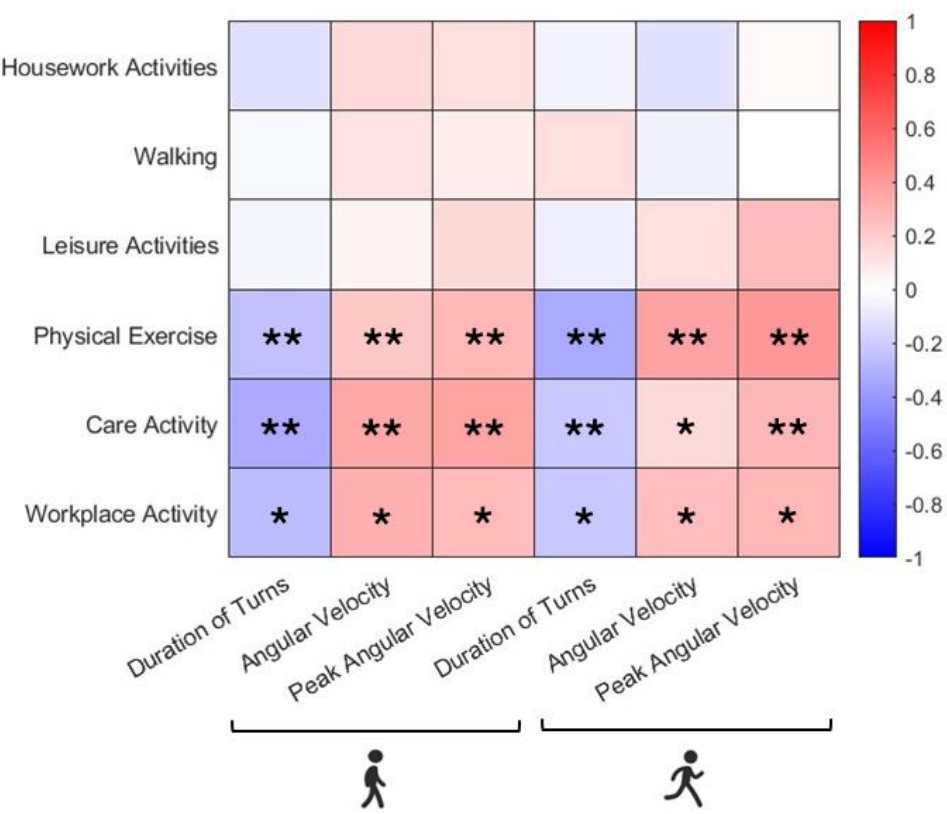
Inertial sensors are valuable tools for detecting subtle changes in motor performance, such as turning duration and angular velocity, that are not captured by simply measuring the time to complete the TUG test. This offers a more nuanced approach to evaluating the impact of motor reserve on mobility changes in PD.

The study entails some limitations. First, the sample size may be too small to consider all confounding factors. Second, we were not able to include an unsupervised assessment, as the features related to bradykinesia are expected to be more evident in the home-setting compared to supervised evaluation in the clinic [21]. Still, the data showed an important difference of turning time and speed even in the absence of changes in total TUG time, thus confirming the potential usability of this measure as progression marker. One of the most important current limitations in PD research is indeed the lack of digital markers able to track subtle changes of disease progression

at this stage. This issue is highly relevant for the research community, considering the upcoming availability of disease-modifying treatments.

Motor reserve could play a significant role in the mobility and motor performance of patients with Parkinson's disease. Understanding motor reserve can help tailor interventions to individual needs, improving mobility outcomes in PD patients.

Figure 1. Correlations between digital parameters and MRI subitems



Partial correlation adjusted for age and sex during self-selected pace TUG and fast TUG

* $p < 0.05$

** $p < 0.01$

Table 1. Clinical characteristics of the patients

	Low MR	High MR	p-value
Participants [n]	21	21	
Age [years]	68.8±7.7	69.5±7.5	0.571 ^b
Sex [% male]	47.6	28.6	0.204 ^a
Disease duration [years]	2.8±1.4	3.1±1.7	0.732 ^b
MDS-UPDRS III	18.1±7.6	16.7±8.6	0.290 ^b
H&Y stage	2.1±1.1	2.0±1.3	0.731 ^b
MoCA, total score	27.5±4.6	28.7±1.1	0.467 ^b
Education [years]	8.1±2.7	9.3±3.4	0.326 ^b
Height [m]	1.69±0.01	1.64±0.01	0.035^b
BMI	24.9±3.8	24.5±3.6	0.772 ^b

a. Chi-square test

b U Mann-Whitney test

Table 2. Cognitive and Motor Reserve of the patients

	Low MR	High MR	p-value
Participants [n]	25	17	
CRI Education	104.7±13.8	102.5±11.2	0.960 ^a
CRI Work	98.9±17.8	99.8±17.6	0.514 ^a
CRI Leisure	105.1±16.0	107.8±19.5	0.151 ^a
CRI Total Score	103.6±17.0	104.3±12.9	0.221 ^a
MRI Attività domestiche	14.0±11.5	28.1±18.2	0.017^a
MRI Spostamento a piedi	7.9±6.4	21.7±20.2	0.079 ^a
MRI Tempo Libero	12.4±7.2	23.4±19.9	0.005^a
MRI Esercizio Fisico	7.0±6.5	16.4±13.0	0.022^a
MRI Attività di cura	14.1±4.2	22.5±10.9	0.022^a
MRI Lavoro	19.1±9.9	22.8±12.3	0.650 ^a
MRI Total Score (%)	12.1±1.8	21.7±9.0	0.000^a

a U Mann-Whitney test

Table 3. Turning characteristics of the patients

TURNING PARAMETERS	Low MR	High MR	p-value
TUG Normal Speed			
TUG Duration [s]	17.8±7.6	16.1±4.7	0.220 ^a
Duration of turns [s]	3.4±0.7	2.7±0.7	0.003^a
Mean Angular Velocity [°/s]	53.7±10.8	67.8±13.7	0.001^a
Peak Angular Velocity [°/s]	119.6±20.1	152.6±31.4	0.000^a
TUG Fast Speed			
TUG Duration [s]	14.0±5.9	12.7±3.6	0.231 ^a
% Variation Tug Duration	-27.9±11.0	-27.1±9.8	0.748 ^b
Duration of turns [s]	2.8±0.8	2.3±0.5	0.034^a
% Variation Duration of Turns	-20.9±19.3	-16.0±14.2	0.475 ^b
Mean Angular Velocity [°/s]	68.2±16.7	77.0±14.6	0.024^a
% Variation Mean Angular Velocity	17.9±9.1	11.8±11.3	0.125 ^b
Peak Angular Velocity [°/s]	161.6±39.8	184.0±37.7	0.020^a
% Variation Peak Angular Velocity	23.6±10.6	17.8±10.4	0.271 ^b

a Parametric comparison corrected for age, sex and UPDRS III

b Non-parametric comparison corrected for age, sex and UPDRS III

(TUG Timed up and go test)

Supplementary Table 1. Turning characteristics of the subjects

TURNING PARAMETERS	HC	PD	p-value
Participants (n)	40	42	
TUG Normal Speed			
TUG Duration [s]	11.3±2.1	17.8±7.8	0.001^a
Duration of turns [s]	2.3±0.4	3.0±0.7	0.003^a
Mean Angular Velocity [°/s]	82.0±14.1	60.9±12.8	0.001^a
Peak Angular Velocity [°/s]	194.3±34.2	136.6±26.4	0.001^a
TUG Fast Speed			
TUG Duration [s]	8.9±1.8	13.3±4.2	0.001^a
% Variation Tug Duration	-21.8±8.8	-26.1±9.8	0.042^b
Duration of turns [s]	1.9±0.3	2.6±0.6	0.014^a
% Variation Duration of Turns	-16.6±12.6	-18.0±16.2	0.315 ^b
Mean Angular Velocity [°/s]	98.2±18.1	73.3±15.2	0.001^a
% Variation Mean Angular Velocity	20.9±16.4	11.8±11.3	0.045^b
Peak Angular Velocity [°/s]	235.4±39.8	172.0±38.4	0.001^a
% Variation Peak Angular Velocity	22.6±13.2	20.8±10.4	0.471 ^b

a Parametric comparison corrected for age, sex

b Non-parametric comparison corrected for age, sex

(TUG Timed up and go test)

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3. CONCLUSION

The present doctoral research has demonstrated the potential of biological and digital markers to monitor and assess non-motor symptoms such as fatigue, sleep disturbances, and activities of daily living in neurodegenerative diseases. Through the integration of advanced mobile health technologies (MHT), wearable devices and AI-driven algorithms, this work has provided novel insights into early detection, personalized interventions and disease progression tracking. The results contribute to the existing literature by bridging the gap between traditional clinical assessments and real-world data collection, emphasizing the importance of ecologically valid and continuous monitoring approaches to provide a more comprehensive understanding of disease burden and progression.

The findings highlight the value of digital solutions in addressing current limitations in clinical practice, offering tools for more accurate, accessible and patient-centered care. For instance, the use of wearable sensors and AI-based analytics has shown promise in identifying subtle motor and non-motor changes that are often missed in routine clinical evaluations.

Clinical Applicability and Integration into Routine Care

The integration of these digital tools into routine clinical care could rearrange the management of neurodegenerative diseases. Wearable devices, for example, could be used to monitor sleep patterns, fatigue levels and daily activities in real-time, allowing clinicians to tailor interventions based on objective, longitudinal data. This could lead to earlier detection of symptom exacerbations, more personalized treatment plans and improved patient outcomes. Additionally, the development of standardized protocols for data collection and analysis could facilitate the adoption of these technologies in clinical practice. A digital toolkit could be developed to include wearable sensors for continuous monitoring, AI-driven algorithms for real-time data analysis,

patient-friendly mobile applications for feedback and adherence and clinician dashboards for visualizing patient data and tracking disease progression over time. Such a toolkit could be integrated into existing healthcare systems enabling seamless data sharing between patients and clinicians. This would not only enhance the quality of care but also reduce the burden on healthcare providers by automating data collection and analysis.

Future Research Directions

While this work has tried to lay a foundation for the use of digital biomarkers in neurodegenerative diseases several areas warrant further investigation. Future research should focus on validating these findings in larger, multicentric cohorts to ensure their generalizability and reliability.

Expanding the scope of studies to include several patient populations and longer follow-up periods will be critical for establishing the robustness of these tools. Additionally, the standardization of data collection and analysis protocols will be essential for ensuring consistency and comparability across studies. Combining digital biomarkers with other modalities, such as neuroimaging and genetic data, could provide a more holistic understanding of disease mechanisms and progression. Conducting randomized controlled trials to evaluate the efficacy of digital tools in improving patient outcomes such as quality of life, symptom management and disease progression will be essential for their adoption in clinical practice. Furthermore, leveraging machine learning and AI to develop predictive models for disease progression and treatment response could enhance the clinical utility of these technologies.

Recommendations for Comprehensive Assessment

Based on the findings of this PhD work, several recommendations can be proposed for the comprehensive assessment of motor and non-motor symptoms in neurodegenerative diseases.

First, adopting a multimodal approach that combines mobile health technologies and traditional clinical assessments can provide a more comprehensive picture of disease burden. Second, prioritizing the collection of data in naturalistic settings will help better understand the impact of symptoms on daily life. Third, establishing guidelines for the use of digital tools in clinical practice, including data collection, analysis, and interpretation, will be critical for their widespread adoption. Fourth, using digital tools to empower patients by providing them with real-time feedback and personalized recommendations can promote patient-centered care. Finally, fostering collaboration between clinicians, researchers, and technology developers will accelerate the translation of digital biomarkers into clinical practice.

In conclusion, this doctoral research has tried to demonstrate the potential of digital biomarkers to transform the assessment and management of neurodegenerative diseases. By addressing the limitations of traditional clinical assessments and leveraging the power of continuous, real-world data collection, these tools offer new opportunities for early detection, personalized care, and improved patient outcomes. Future research and clinical trials will be critical for validating these findings and translating them into routine clinical practice. With the development of standardized protocols and digital toolkits, the integration of these technologies into healthcare systems could pave the way for a new era of patient-centered, data-driven care in neurodegenerative diseases.