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Functional impact on gait and balance in Parkinson's disease using advanced technologies

Marta Francisca Corrà

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Marta Francisca Corrà

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I dedicate my thesis to my friends and to my big and loud Italian family.

List of Publications

Publications as first author included in this thesis:

1. **Corrà MF**, Warmerdam E, Vila-Chã N, Maetzler W, Maia LF. Wearable Health Technology to Quantify the Functional Impact of Peripheral Neuropathy on Mobility in Parkinson's Disease: A Systematic Review. Sensors (Basel). 2020 Nov 19;20(22):6627. doi: 10.3390/s20226627.

2. **Corrà MF**, Atrsaei A, Sardoreira A, Hansen C, Aminian K, Correia M, Vila-Chã N, Maetzler W, Maia LF. Comparison of Laboratory and Daily-Life Gait Speed Assessment during ON and OFF States in Parkinson's Disease. Sensors (Basel). 2021 Jun 9;21(12):3974. doi: 10.3390/s21123974.

3. **Corrà MF**, Sousa M, Reis I, Tanganelli F, Vila-Chã N, Sousa AP, Magalhães R, Sampaio P, Taipa R, Maia LF. Advantages of an Automated Method Compared With Manual Methods for the Quantification of Intraepidermal Nerve Fiber in Skin Biopsy. J Neuropathol Exp Neurol. 2021 May 26:nlab045. doi:10.1093/jnen/nlab045.

4**. Corrà MF**, Vila-Chã N, Sardoreira A, Hansen C, Sousa AP, Reis I, Sambayeta F, Damásio J, Calejo M, Schicketmueller A, Laranjinha I, Salgado P, Taipa R, Magalhães R, Correia M, Maetzler W, Maia LF. Peripheral neuropathy in Parkinson's disease: prevalence and functional impacto on gait and balance. Brain. 2022 Jan 28:awac026. doi: 10.1093/brain/awac026. Epub ahead of print. PMID: 35088837.

Other publications as co-author:

1. Geritz J, Maetzold S, Steffen M, Pilotto A, **Corrà MF**, Moscovich M, Rizzetti MC, Borroni B, Padovani A, Alpes A, Bang C, Barcellos I, Baron R, Bartsch T, Becktepe JS, Berg D, Bergeest LM, Bergmann P, Bouça-Machado R, Drey M, Elshehabi M, Farahmandi S, Ferreira JJ, Franke A, Friederich A, Geisler C, Hüllemann P, Gierthmühlen J, Granert O, Heinzel S, Heller MK, Hobert MA, Hofmann M, Jemlich B, Kerkmann L, Knüpfer S, Krause K, Kress M, Krupp S, Kudelka J, Kuhlenbäumer G, Kurth R, Leypoldt F, Maetzler C, Maia LF, Moewius A, Neumann P, Niemann K, Ortlieb CT, Paschen S, Pham MH, Puehler T, Radloff F, Riedel C, Rogalski M, Sablowsky S, Schanz EM, Schebesta L, Schicketmüller A, Studt S, Thieves M, Tönges L, Ullrich S, Urban PP, Vila-Chã N, Wiegard A, Warmerdam E, Warnecke T, Weiss M, Welzel J, Hansen C, Maetzler W. Motor, cognitive and mobility

deficits in 1000 geriatric patients: protocol of a quantitative observational study before and after routine clinical geriatric treatment - the ComOn-study. BMC Geriatr. 2020 Feb 6;20(1):45. doi: 10.1186/s12877-020-1445-z.

2. Atrsaei A, **Corrà MF**, Dadashi F, Vila-Chã N, Maia L, Mariani B, Maetzler W, Aminian K. Gait speed in clinical and daily living assessments in Parkinson's disease patients: performance versus capacity. NPJ Parkinsons Dis. 2021 Mar 5;7(1):24. doi: 10.1038/s41531-021-00171-0.

Abstract

The clinical features of Parkinson's disease (PD) appear gradually and are usually divided into motor and non-motor symptoms, which together lead to a considerable and complex disability. Precise assessment and characterization of all PD hallmarks are critical to monitor disease progression and adapt ongoing treatments. The use of wearable health-technology in clinical research has provided faster and easier monitoring of PD motor symptoms, but has also brought attention to some discrepancies between assessments in different environments.

Among the major clinical features of PD, peripheral neuropathy (PNP) has been increasingly recognized as a common problem in PD. PNP is a peripheral nervous system disorder with a wide spectrum of clinical manifestations, which can worsen the overall mobility of PD patients. However, its functional impact on gait and balance in PD has never been extensively investigated.

In light of this evidence, this study was divided into three parts. The first aim of the project was to use wearable health-technology to compare the mobility of patients with PD in different settings, such as the laboratory and at home. The goal of this first part was to expand information about daily-life performance and improve clinical care with targeted interventions.

The second aim of the study was to investigate the functional impact of PNP on gait and balance in PD, using the same wearable health-technology. A systematic review was elaborated to examine the most relevant and clinically useful methodologies for characterizing gait and balance deficits in PD and PNP with wearables. Subsequently, the same proposed indications were used to study the effect of PNP on mobility in a consecutive cohort of PD patients. In order to characterize PNP in PD, we performed a comprehensive assessment that included detailed clinical scales, neurophysiological and neuropathological examinations, the latter consisting of manual counting of small nerve fibers from skin biopsies' punches.

As a final part of the project, to overcome the main limitations of the manual quantification of small nerve fibers in skin biopsies, which were also confirmed during the evaluation of PNP in our PD cohort, we developed a new automated method for small nerve fibers quantification for application in research and clinical settings.

The first aim of the study was achieved by assessing a total of 27 patients with PD in their OFF and ON medication states with two different gait assessments, which included several gait tasks in the laboratory and an unsupervised full-day assessment at home. During ON

medication state, we observed that gait speed assessed at fast pace in the laboratory showed the highest association with home gait speed, and, in contrast, home gait speed showed lower correlations with normal pace walking in the laboratory. This suggests that assessing higher speeds and maximum capacity in the laboratory may provide more information about PD performance in real life. With regard to other tasks such as circular walking, we found that this type of gait task reliably represented the speed of a patient's daily gait at home. This analysis demonstrated that clinicians can tailor gait assessments and monitor patients by performing gait tasks under different conditions, covering a wider range of gait speeds values that are more similar to patients' real-life motor status.

The second aim was achieved by investigating the functional impact of PNP on gait and balance in a cohort of PD patients. We first defined a customized assessment protocol for evaluating PNP in PD with wearable health-technology, which included the number and location of wearable sensors on the body, the type of tasks to perform and the parameters to be extracted to best differentiate PNP in PD patients. In light of these indications, a total of 99 consecutive PD patients from Movement Disorders' consultations were assessed, and PNP was defined based on comprehensive diagnostic criteria, with no restriction on PNP type. We found that PNP was common in PD (40.4% of the cohort), and had a functional impact on gait and balance. We observed a more impaired gait in PD patients with PNP during all gait tasks, which was the result of the contribution of the neuropathic motor, sensory and proprioceptive deficits. PNP also had a significant impact on balance: postural instability was more evident during more challenging tasks and in anterior-posterior (AP) sway directions, suggesting that PNP may predominantly show hip strategy to compensate for the balance deficits.

In conclusion, we confirmed that wearable health-technology can provide a great advantage when assessing gait and balance deficits of PNP in PD, because they allow for the detection of changes that would be poorly perceived with other tools. Recognizing and assessing PNP in PD is therefore critical to improve PD patients' gait and minimize balance deficits, and to target individualized medical care. The accurate quantification of different parameters may also raise the possibility to establish new cut-offs for the characterization of gait and balance in this specific subset of patients.

Finally, the third aim of the project was achieved by developing a new automated counting method to detect small nerve fibers in skin biopsies. A total of 60 skin biopsy specimens from our pool of patients were used to compare the new automated method with manual counting performed by three independent observers on the same samples. We obtained significant reliability of measurements and faster and a more standardized procedure, which eliminated the long counting times and the higher interrater variability typical of the manual quantification. The moderate-to-high association between classical and automated counting

demonstrated its possible applicability in clinical settings and use for the diagnosis of small-fiber neuropathy.

In conclusion, the study of PD with the use of newer and more advanced technologies, the monitoring of PD motor function in different environments, and the deeper exploration of PD features such as PNP can lead to more accurate patient stratification, more personalized interventions and treatment optimization, with the ultimate goal of increasing patients' quality of life.

Resumo

As características clínicas da doença de Parkinson (DP) aparecem gradualmente e dividem-se geralmente em sintomas motores e não motores, que juntos levam a uma incapacidade considerável e complexa. A avaliação precisa e a caracterização de todas as características da DP são fundamentais para monitorizar a progressão da doença e adaptar os tratamentos em curso. A utilização de tecnologias de saúde vestíveis na investigação clínica tem proporcionado uma monitorização mais rápida e fácil dos sintomas motores da DP, mas também chamou a atenção para algumas discrepâncias entre as avaliações em diferentes ambientes.

Entre as principais características clínicas da DP, a neuropatia periférica (PNP) tem sido cada vez mais reconhecida como um problema comum na DP. A PNP é uma doença do sistema nervoso periférico com um vasto espectro de manifestações clínicas, que pode agravar a mobilidade global dos doentes de DP. No entanto, o seu impacto funcional na marcha e equilíbrio na DP nunca foi extensivamente investigado.

Á luz destas evidências, este estudo foi dividido em três partes. O primeiro objetivo do projeto era utilizar tecnologias de saúde vestíveis para comparar a mobilidade dos pacientes com DP em diferentes ambientes, tais como o laboratório e em casa. O objetivo desta primeira parte era expandir a informação sobre o desempenho da vida diária e melhorar os cuidados clínicos com intervenções orientadas.

O segundo objetivo do estudo era investigar o impacto funcional da PNP na marcha e equilíbrio na DP, utilizando a mesma tecnologia de saúde vestível. Foi elaborada uma revisão sistemática para examinar as metodologias mais relevantes e clinicamente úteis para caracterizar os défices de marcha e equilíbrio na DP e PNP com tecnologias vestíveis. Subsequentemente, as mesmas indicações propostas foram utilizadas para estudar o efeito do PNP na mobilidade numa coorte consecutiva de pacientes com DP. A fim de caracterizar o PNP na DP, realizámos uma avaliação abrangente que incluiu escalas clínicas detalhadas, exames neurofisiológicos e neuropatológicos, este último consistindo na contagem manual de pequenas fibras nervosas a partir de punções de biópsias de pele. Como parte final do projeto, para superar as principais limitações da quantificação manual de pequenas fibras nervosas em biópsias de pele, que também foram confirmadas durante a avaliação do PNP na nossa coorte de DP, desenvolvemos um novo método automatizado de quantificação de pequenas fibras nervosas para aplicação em ambientes clínicos e de investigação.

O primeiro objetivo do estudo foi alcançado através da avaliação de um total de 27 pacientes com DP nos seus estados de medicação OFF e ON com duas avaliações de marcha diferentes, que incluíram várias tarefas de marcha no laboratório e uma avaliação não supervisionada de dia inteiro em casa. Durante o estado de medicação ON, observámos que a velocidade de marcha avaliada em ritmo acelerado no laboratório mostrou a maior associação com a velocidade de marcha em casa, e, em contraste, a velocidade de marcha em casa mostrou correlações mais baixas com o ritmo normal de marcha no laboratório. Isto sugere que a avaliação de velocidades mais elevadas e capacidade máxima no laboratório pode fornecer mais informação sobre o desempenho da DP na vida real. Em relação a outras tarefas como a marcha circular, descobrimos que este tipo de tarefa de marcha representava de forma fiável a velocidade da marcha diária de um paciente em casa. Esta análise demonstrou que os clínicos podem adaptar as avaliações da marcha e monitorizar os pacientes realizando tarefas de marcha em diferentes condições, cobrindo uma gama mais ampla de valores de velocidade de marcha que são mais semelhantes ao estado motor da vida real dos pacientes.

O segundo objetivo foi alcançado através da investigação do impacto funcional do PNP na marcha e equilíbrio numa coorte de pacientes com DP. Primeiro definimos um protocolo de avaliação personalizado para avaliar a PNP em DP com tecnologias de saúde vestíveis, que incluía o número e a localização de sensores desgastáveis no corpo, o tipo de tarefas a realizar e os parâmetros a extrair para melhor diferenciar a PNP em pacientes com DP. À luz destas indicações, foi avaliado um total de 99 pacientes com DP consecutivos das consultas do movimento, e o PNP foi definido com base em critérios de diagnóstico abrangentes, sem qualquer restrição do tipo de PNP. Verificámos que a PNP era comum na DP (40,4% da coorte), e teve um impacto funcional na marcha e equilíbrio. Observámos uma marcha mais prejudicada em pacientes DP com PNP durante todas as tarefas de marcha, que foi o resultado da contribuição dos défices motores neuropáticos, sensoriais e propriocetivos. A PNP também teve um impacto significativo no equilíbrio: a instabilidade postural foi mais evidente durante as tarefas mais desafiantes e nas direções de oscilação ântero-posterior (AP), sugerindo que a PNP pode mostrar predominantemente uma estratégia da anca para compensar os défices de equilíbrio.

Em conclusão, confirmámos que a tecnologia de saúde desgastável pode proporcionar uma grande vantagem ao avaliar os défices de marcha e equilíbrio do PNP na DP, porque permite a deteção de alterações que seriam mal percebidas com outras ferramentas. O reconhecimento e a avaliação do PNP na DP é, portanto, fundamental para melhorar a marcha dos pacientes com DP e minimizar os défices de equilíbrio, e para visar cuidados médicos individualizados. A quantificação precisa de diferentes parâmetros pode também

levantar a possibilidade de estabelecer novos *cut-offs* para a caracterização da marcha e do equilíbrio neste subconjunto específico de pacientes.
Finalmente, o terceiro objetivo do projeto foi alcançado através do desenvolvimento de um novo método de contagem automatizado para detetar pequenas fibras nervosas nas biópsias de pele. Um total de 60 amostras de biopsias de pele do nosso conjunto de pacientes foi utilizado para comparar o novo método automatizado com a contagem manual realizada por três observadores independentes sobre as mesmas amostras. Obtivemos uma fiabilidade significativa das medições e um procedimento mais rápido e padronizado, o que eliminou os longos tempos de contagem e a maior variabilidade entre os diferentes intervenientes típica da quantificação manual. A associação moderadamente elevada entre a contagem clássica e automatizada demonstrou a sua possível aplicabilidade em ambientes clínicos e utilização para o diagnóstico de neuropatia de pequenas fibras.

Em conclusão, o estudo da DP com o uso de tecnologias mais recentes e avançadas, a monitorização da função motora da DP em diferentes ambientes, e a exploração mais profunda de características da DP, tais como a PNP, pode levar a uma estratificação mais precisa do paciente, intervenções mais personalizadas e otimização do tratamento, com o objetivo final de aumentar a qualidade de vida dos pacientes.

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List of abbreviations

3D	3-Dimension
ACC	Accelerometer
ADL	Activities of Daily Living
AIL	Automated intradermal line
AP	Anterior-posterior
BBS	Berg Balance Scale
BMI	Body Mass Index
CCF	Cross-correlation function
CIDN	Chronic inflammatory demyelinating polyneuropathy
CIPN	Chemotherapy-induced demyelinating polyneuropathy
CMC	Classic Manual Counting
COG	Center of Gravity
COM	Center of mass
COP	Center of pressure
CV	Coefficient of variation
DBS	Deep Brain Stimulation
DFU	Diabetic foot ulcer
DM	Diabetes Mellitus
DPN	Diabetic peripheral neuropathy
DRS	Dementia rating scale
EMG	Electromyography
FAB	Fullerton Advance Balance test
FIAC	Fluorescence Images Automated Counting
FIMC	Fluorescence Images Manual Counting
FOG	Freezing of Gait
GYR	Gyroscope
HC	Healthy controls
Hcy	Homocysteine
H&Y	Hoehn and Yahr
ICC	Interclass correlation coefficient
IENF	Intraepidermal Nerve Fiber
IMU	Inertial Measurement Unit
iTUG	Instrumented Timed up-and-go test
L-dopa	Levodopa
LB	Lewy Bodies
LEDD	Levodopa daily dose
MAG	Magnetometer
MIL	Manual intradermal line

MMA	Methylmalonic acid
MeSH	Medical Subject Headings
ML	Mediolateral
MNSI	Michigan Neuropathy Screening Instrument
mTCNS	Modified Toronto Clinical Neuropathy Score
NCS	Nerve conduction studies
NDS	Neuropathy Disability Score
NeP-DPN	Neuropathic pain diabetic neuropathy
NIS-LL	Neuropathy Impairment Score for Lower Limbs
NMSS	Non-motor symptoms scale
NSP	Neuropathy Symptoms profile
NSS	Neuropathy Symptoms Score
PD	Parkinson's Disease
PD-noPNP	Parkinson's disease without peripheral neuropathy
PD-PNP	Parkinson's disease with peripheral neuropathy
PDQ	Parkinson's disease questionnaire
PGP 9.5	Protein Gene Product 9.5
PNP	Peripheral Neuropathy
PNP-LL	Peripheral Neuropathy of the lower limbs
PNS	Peripheral Nervous System
QST	Quantitative Sensory Testing
RBD	REM Sleep Behavior Disorder
REM	Rapid Eye Movement
RMS	Root mean square
ROI	Region of interest
SFN	Small fiber neuropathy
SOT	Sensory organization test
TBS	Tinetti Balance Scale
TCNS	Toronto Clinical Neuropathy Score
TCS	Total Neuropathy Score
TUG	Timed Up and Go
UENS	Utah Early Neuropathy Scale
UPDRS	Unified Parkinson's Disease Rating Scale
VB12	Vitamin B12
WB	Walking bouts

CHAPTER 1.
INTRODUCTION

PARKINSON'S DISEASE

Parkinson's disease (PD) is a complex neurodegenerative disease affecting the Central Nervous System, with gradual and prolonged progression. As the disease progresses, symptoms usually emerge slowly, and lead to a gradual deterioration in activities of daily-living [1, 2]. It was firstly described by James Parkinson in 1817, and later re-defined and differentiated from other neurological conditions by Jean-Martin Charcot, who distinguished its cardinal features [3].

PD is the second most common neurodegenerative disease, with an increasing incidence with age in both women and men [4]. It has a prevalence of 0.3% in the entire population and of about 1% in persons over 60 years of age in industrialized countries. In Europe, the prevalence is about 3.5% in population of 85-89 years of age [1, 5].

At neuropathological level, PD is characterized by the progressive death of pigmented dopaminergic neurons in the nigrostriatal system, and in particular in the substantia nigra parts compact, in the midbrain. Lesions of dopaminergic neurons result in degeneration of the nigrostriatal pathway [6], and subsequent deposition of phosphorylated α-synuclein aggregates, the major protein marker and biological hallmark of PD [7]. These aggregates form intra-cytoplasmatic inclusions called Lewy bodies (LB), which become misfolded and accumulate in numerous organs and body systems, such as spinal cord and peripheral nervous system, salivary glands and cardiac plexus (Fig. 1) [8-11]. As reported by Braak et al [12], LB inclusions are progressively present in the olfactory bulb before clinical symptoms occur, then affect the midbrain and forebrain, and in later stages the degeneration process enters the neocortex with a wide variety of clinical manifestations. This model proposed by Braak has received considerable attention because the temporal- and spatial-progression of neuronal degeneration seems to explain the clinical course of PD [11]. In particular, clinical features of PD appear gradually and are usually divided into motor and non-motor symptoms, encompassing several functions of the nervous system, all of which together represent a considerable complex disability [1, 13].

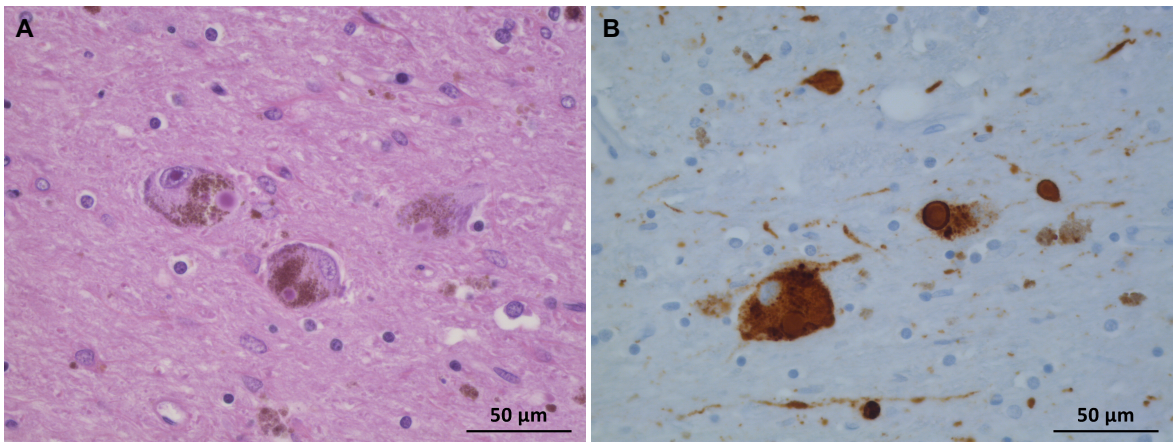

Figure 1. Neuronal loss of substantia nigra with Lewy bodies in the remaining neurons. **(A)** H&E; **(B)** α-synuclein immunohistochemistry, clone KM51 antibody (Leica, UK). Courtesy of Portuguese Brain Bank, Centro Hospitalar Universitário do Porto (CHUP).

MOTOR SYMPTOMS

The effects of PD can be described at several levels. Motor symptoms are usually the first identifiers, and appear during the entire course of the disease [14]. According to the Movement Disorder Society, the three main motor symptoms in PD are bradykinesia, resting tremor and rigidity, which usually begins unilaterally with asymmetric effects [9].

Bradykinesia is slowness of movements, and is defined as the hallmark of basal ganglia disorders [2]. It refers to the difficulty in initiating and executing movements, especially when performing sequential and simultaneous tasks. It may first appear before any formal neurological examination, and is often characterized by difficulty in performing activities of daily living and slower movement reaction times [15].

Resting tremor usually starts unilaterally, and it is more evident in the distal parts of the body [16]. The occurrence of tremor in PD has not been extensively investigated, and it varies during the course of the disease: in a study of 100 cases, approximately 69% of patients with PD showed resting tremor at the disease onset, 75% during the course of the disease, and in 9% of patients tremor disappeared at later stages [11, 17]. Tremor usually disappears with action and during sleep, which allows it to be differentiated from essential tremor [18]. In some patients, resting tremor is perceived as 'internal' shaking [2].

Finally, rigidity or stiffness refers to a continuous and uniform increase in muscle tone, perceived as constant resistance to passive movement [19]. It can occur both proximally and distally, and in the early stages of the disease: complaints of stiffness, tremors and imbalance have been shown to be associated with an increased risk of future falls in PD [20]. Rigidity can also result in abnormal postural deformities, especially when it affects the neck and truck (axial rigidity), but these deformities generally occur later in the course of

the disease [2]. It can also result in a sudden akinesia, which is the complete loss and inability to move, also called as freezing of gait (FOG). Because of the hesitation when beginning to walk and to move the feet, FOG is one of the most disabling symptoms. It leads to significant social and clinical consequences, and is a common cause of falls in PD patients [21].

Other motor complications in PD include gait and speech difficulties, hypophonia and general postural instability, which usually appear at later stages of the disease [9].

All of these motor symptoms and in particular postural instability and FOG significantly contribute to increased risk of falls. Falls are a major complaints among patients with PD and are usually rare during the first years after disease onset [2]. However, most patients experience current falls during the course of the disease: five prospective surveys of PD patients showed that nearly 70% of patients fall, and recurrent falls occurred in approximately 50% of patients during 1 year follow-up [21]. Falls can result from changes in posture, rotational movements, and concurrent activities, but may also have other risk factors such as orthostatic hypotension and dementia [22].

Falls and mobility problems generate a vicious cycle that has a devastating impact on patients' social activities and quality of life: reduced mobility due to fear of falling causes loss of independence and deprives patients of social contacts. In addition, prolonged periods of immobility result in other side effects such as depression, osteoporosis, reduced overall physical fitness, increased cardiovascular morbidity, and finally a higher risk of mortality [13, 21, 22]. Therefore, detailed and personalized management of PD symptoms and progression, and the investigation of further treatments and strategies to delay disability are the main goals to be addressed in current and future research to improve the quality of life of people with PD.

MANAGEMENT OF MOTOR SYMPTOMS

PD is still not treatable. Available pharmacological and non-pharmacological treatments do not modify the neurodegenerative progression of the disease, but can only provide symptomatic relief and improve the patient's functional capacity for as long as possible [23]. For this reason, a large portion of research in recent decades is increasingly focusing attention on finding new and more effective agents [9].

Available therapies used to treat PD have two main modes of action: increasing the level of dopamine in the brain, or mimicking its effect [23]. In particular, levodopa (L-dopa) is the acting precursor of dopamine and the key pharmacological compound in the treatment of PD symptoms [24]. Although the use of dopamine-based therapies has been highly

successful in reducing many of the motor symptoms in PD, additional motor complications are known to arise from long-term use of these drugs [25]. Prolonged treatment with L-dopa is associated with several adverse motor effects, such as dyskinesia, ON-OFF phenomena and wearing OFF effects [26]. In particular, dyskinesias are involuntary and erratic body movements that occur in approximately 30-80% of patients treated with L-dopa for at least 3 years [27], probably due to neuronal degeneration of L-dopa on neurons due to long-term effects [28]. In some cases, dyskinesias are experienced at times of transition to an ON phase [24]. Another long-term effect is the fluctuation in drug response, also called as ON-OFF phenomenon. After approximately 4-6 years of dopaminergic therapy, the effect of L-dopa starts to shorten, and so-called OFF periods become more extensive over time [29]. These periods are characterized by severe akinesia that can last for many hours. The incidence of ON-OFF phenomena and fluctuations in drug response is influenced by the daily duration of L-dopa therapy, which commonly requires periodic adjustments to achieve an optimal response-to-drug window. Relapses and subsequent fluctuations are very common, and beneficial effects are often short-lived [23].

Although L-dopa is the most effective drug in improving PD symptoms [9], thanks to the understanding of the nigrostriatal dopaminergic transmission (and, consequently, its multiple additional therapeutic targets) new several approaches have recently been investigated [24]. In particular, newer dopaminergic agonists have been shown to produce a lower incidence of involuntary movements, with a longer duration of action [25]. Other approaches that focus on non-dopaminergic systems have been designed to improve motor function without the risk of motor complications, such as surgical and behavioral rehabilitation therapies. For example, the introduction of deep brain stimulation (DBS) represents a major breakthrough and innovative expansion in the treatment of PD. There is still a need to further explore other treatments, and to support patients-specific care that is effective and with as few side effects as possible.

Even with medical (and surgical) treatments, PD symptoms may not be fully controlled. Adverse side effects and limited treatment efficacy require clinicians and healthcare providers to continuously monitor PD symptoms and disease progression over time with specific tools. There are several approaches to monitoring therapeutic interventions and PD progression. One of the most applied methods in clinical practice is the use of rating scales. [30]. Generally, rating scales can be divided in those that are more specific for the assessment of disease progression and motor fluctuations [31, 32] and those that provide a general level of motor function, requested directly to the patient or caregiver (e.g., the

Schwab and England ADL scale) [33]. Other types of rating scales include those that assess psychiatric manifestations (for example depression) and quality of life.

The Unified Parkinson's Disease Rating Scale (UPDRS, and the Movement Disorder Society-revised version thereof) is a validated clinical scale that specifically assesses PD disability and impairment, including both historical information and clinical examination [34]. The UPRDS is an effective measurement tool: with the aim of investigating and tracking PD symptoms and progression, a study on the use of UPDRS confirmed that PD has a non-linear course, with a variable rate of deterioration, more rapid in the early stage of the disease and in patients with greater postural instability or gait difficulties [35]. Another study demonstrated the effectiveness of assessments of different medication states: an annual rate of decline in the total UPDRS scores of 1.34 points when assessed during ON state, and of 1.58 points when assessed during OFF medication states was observed [2]. Older patients usually experienced significantly more disease progression in mental activity and FOG rates of the UPDRS. Many other studies have demonstrated the benefits of using rating scales in the management of PD, for example in the analysis of pharmacological complications [36], or in the assessment of motor fluctuations [37].

However, clinical scores do not provide objective data that are representative of the patient's health status. It has been observed that measuring motor signs with UPDRS may not be optimal especially in the early stages of PD, because many items were proposed to specifically evaluate more advanced features of the disease [38]. The same study also addressed some psychometric issues regarding the subjectivity of the scale and the modest reliability. The use of objective measures of movement to track disease progression and severity could compensate for these subjective limitations, and allow for greater accuracy and reproducibility.

WEARABLE HEALTH-TECHNOLOGY

Because of the need for more objective measures to quantify and monitor motor symptoms in PD, the use of smart technologies has significantly increased in recent years. In particular, wearable health-technologies have been of fundamental importance in helping clinicians provide unbiased measurements that can be used in both daily clinical practice and scientific research [39]. Wearables are mobile devices worn on the body, such as inertial measurement units (IMUs), smartwatches, or Holter electrocardiogram monitors [40]. Specifically, IMUs typically consist of accelerometers, which determine the acceleration, and gyroscopes, which measure angular velocities. In several types of IMUs, magnetometers are added to measure magnetic fields [41] (Fig. 2). The main advantages

of IMUs are the small size and the low costs of their components, compared to other devices for movement analysis. IMUs are easy to use and thus can simplify data management and patient participation [39]. In addition, compared with other types of non-invasive sensors used for gait assessment (3D motion capture analysis systems, gait mats, or force plates), they are lighter, less time-consuming and do not require specifically equipped laboratories and clinical environments [41, 42]. IMUs can be easily worn on different body segments, via elastic belts or tape bands. The number and location of IMUs depend on the application considered and on the type of assessment performed [42].

IMUs allow the assessment of motor functions, both in healthy subjects and in patients with neurological diseases, and the highly accurate estimation of spatial-temporal and kinematic motor parameters [41]. Many studies have recently adopted wearable health technology in the investigation clinical manifestations of PD. Wearables have demonstrated their potential by improving the sensitivity of clinical tests, such as the Timed Up and Go (TUG) test, or other functional tests, by incorporating IMUs to provide more objective and continuous measures of mobility [43-45]. Many studies have also used wearable health-technology to detect differences in gait and balance between groups: for example PD and healthy controls [44, 45], fallers and non-fallers [46], different PD subtypes, and patients at early stage of PD and people with high risk of developing PD [47-49]. Wearable sensors have been also used to detect various motor symptoms during motor fluctuations [37, 50, 51], and to study the effects of dopaminergic treatment and dyskinesias [52, 53].

Because of the inherent characteristics of being portable and easy to wear, wearables can objectively analyze symptoms and disease progression in controlled settings (such as the clinic or hospital), but also quantify specific motor activities of daily-life under unsupervised conditions, providing the development of more accurate treatment plans than [54-56]. When investigating PD characteristics under unsupervised conditions, it has been shown that motor function observed in supervised settings often do not reflect performances in daily life [57]. This discrepancy may be due to the fact that daily motor activities may be influenced by all measures of daily life that cannot be captured during a supervised assessment: cognitive function, environment, social interactions, and the tendency to change behavior due to awareness of being observed [40]. It remains unclear whether patients' performance in different medication states during clinical evaluation accurately corresponds to actual performance in daily life. Therefore, improving understanding of motor conditions with additional (more ecological) information about motor activities occurring in daily life is essential to improve care and timely identification of pharmacological response throughout the day, ensure faster follow-ups, and overcome the limitations of rating scales.

Figure 2. An example of Inertial Measurement Unit (IMU) worn on the right foot (Hasomed GmbH, Magdeburg, Germany)

NON-MOTOR SYMPTOMS

The traditional view of PD as a motor-symptoms disorder has been changed in recent decades due to a strong clinical and neuropathological evidence of systemic involvement [58]. Symptoms other than motor symptoms have often being under-reported, under-recognized or untreated, since motor complications were traditionally viewed as primary and early identifiers of PD [59]. Motor and non-motor symptoms together have a broader effect on the overall health of patients with PD and can significantly compromise daily activities [9, 60]. Only in recent years have specific trials been conducted to monitor and treat non-motor symptoms [61]. More and more clinical trials are including measures to detect the effect of treatments on both motor and non-motor complications in PD: studies on non-motor side effects of antiparkinsonian medications [62], on physical therapy and exercises [63], on multidisciplinary motor and non-motor approaches [64] and on specific treatments for non-motor symptoms [65] are increasing in number, with the goal of delaying the progressive deterioration of quality of life over the course of the disease [24, 66].

Non-motor symptoms may be linked to pathogenesis of PD through association with Lewy bodies pathology, based on the aforementioned Braak pathological staging. Brainstem involvement has a caudal-to-rostral progression, with the eventual involvement of other brain areas (namely diencephalon, basal forebrain, medial temporal lobe structures and finally the cortex). This implicates that the presence of α-synuclein and Lewy bodies outside

the nigrostriatal system may be responsible for non-motor manifestations in the same fashion [67]. The results of several pathological studies have shown a significant association between cognitive impairment and levels of Lewy bodies in the cerebral cortex [68, 69]. In addition, the presence of Lewy bodies also in several other nerve structures such as the skin indicates a likely involvement of other body systems, such as the peripheral nervous system, as part of the pathological process of PD [70]. Further studies are needed to confirm the correlation between non-motor symptoms and the Braak staging system [11].

The main non-motor symptoms that occur in PD can be divided into cognitive and behavioral changes, autonomic dysfunction, sleep disturbances, fatigue and sensation abnormalities [58]. The overall occurrence of these complications is not easy to determine, however, the appearance of one or more non-motor symptoms usually increases as the disease progresses [71].

Depression, psychiatric symptoms and cognitive impairment are the main behavioral changes in PD. Depressive symptoms occur at any time during the course of the disease, and may even precede the appearance of motor symptoms as early prodromal sign of PD [58, 72]. As depression, mild cognitive decline may also already be present, as nearly 25% of patients at early stage of PD already show mild impairments [58, 73]. A comprehensive study by Verbaan et al [74] found that 22% of a cohort of more than 400 PD patients exhibited impaired cognition.

Autonomic complications can result at any stage of the disease, as both the central and peripheral autonomic nervous systems can be affected [75]. At least one autonomic symptom is present in 71% of patients in the early stage, based on a longitudinal study after 3-years follow-up [76]. Orthostatic hypotension is the most common cardiovascular dysfunction, and may be present in 60% of patients [58]. Gastrointestinal disorders, urinary problems and sexual dysfunction may also be present, albeit rarer and at later stages [77].

Disturbed or impaired sleep is also highly prevalent, and can reach an incidence of almost 90% [58]. In studies investigating quality of life in PD, sleep difficulties have been shown to be important predictors of poor quality of life, because they also contribute to poor daytime functioning [78]. Insomnia and REM sleep behavior disorders (RBD) have recently become a research focus, in terms of manifestations and risk factor, because of their occurrence even years before classic motor features emerge [79]. The average latency between the onset of RBD and the appearance of motor symptoms has been shown to be 12-14 years [11]. Some studies suggest that individuals with RBD have approximately an 80-90% risk of eventually developing PD [58].

Finally, sensory disturbances are common in PD, and generally tend to affect the more severely affected side of the body [80, 81]. Among the major abnormalities of sensation,

olfactory disfunction and pain are widely recognized, and severely affect the quality of life of individuals who experience it [11]. Pain has been shown to affect 76% of patients [58], and can be classified as primary pain (pain related to dyskinesias, OFF periods, central pain) and secondary pain (more musculoskeletal, orofacial, limb and abdominal pain) [82]. The pathogenesis of primary pain has not yet been clearly identified. There are two hypotheses related to the causes of pain in PD [83]: abnormal nociceptive and mechanical thresholds, due to abnormalities in central dopaminergic nociceptive processing and sensorimotor integration [84, 85]; or impaired inhibition of the ascending nociceptive pathway due to diencephalon-spinal dysfunction [86].

Sensory alterations in PD also include abnormalities in tactile, thermal and proprioceptive perception [87], but mechanisms underlying these sensory alterations are not yet widely understood. Two main explanations have been proposed. The striatum receives convergent axonal projections from motor and sensory cortical regions, indicating that dopamine loss may affect both motor and sensory processing functions [88], and, consequently, high L-dopa exposures may be a determinant of peripheral alterations [70]. Another explanation could be the intrinsic deterioration of peripheral sensory nerves in PD, related to the presence of α-synuclein aggregates in the peripheral nervous system [70].

In light of these considerations, the current scientific literature has brought new discussions about the increased presence of peripheral neuropathy in patients with PD. Peripheral neuropathy may increase the disability burden of patients, leading to difficult disease management and decreased quality of life.

PERIPHERAL NEUROPATHY

Peripheral neuropathy (PNP) is a disorder of the peripheral nervous system referring to disease of axons and/or myelin in peripheral nerve fibers [89]. It is a common neurological problem, with distal-proximal gradient characterization, involving dysfunctions of the peripheral motor, sensory and autonomic nerves, and can be divided in acute and chronic, based on the temporal evolution of symptoms [90]. Forms of PNP include mononeuropathies, single or multiple with asymmetric damage (in different areas), and polyneuropathies, affecting multiple symmetrical nerves [90].

PNP has two main phenotypes. Large-fiber neuropathy can be axonal, where axons are most commonly affected in proportion to their length; or demyelinating, when the myelin sheath around axons is damaged [91]. It occurs when Aα and Aβ myelinated fibers are affected: these fibers send vibration and joint position sense, and their injury causes numbness, postural instability, distal weakness and muscle atrophy.

In small-fiber neuropathy, unmyelinated C and thinly myelinated Aδ fibers are damaged. These fibers provide thermal and mechanical pain information, and their damage causes burning, tingling, uncomfortable pin and needle sensation, hyperalgesia (increased pain from a stimulus that usually provokes pain), or allodynia (pain due to a stimulus that does not usually cause pain) as the first sensory manifestations [89, 92]. Neuropathic pain is highly disabling and affects approximately 20-30% of patients with PNP [93]. Autonomic symptoms are often underreported and may have cardiovascular, gastrointestinal, urogenital and secretomotor involvements [94].

The prevalence of PNP in the general population is difficult to record, because it is often underreported and underestimated. The overall prevalence of PNP varies between 2.4% and 8% in studies investigating the incidence of PNP in different countries and with different methodologies [95-98]. This wide variation may be due to differences in populations and study designs or to different protocols for assessing PNP [97]. For example, several studies on PNP prevalence included only symptomatic patients or used a less extensive evaluation of PNP. For this reason, it is worth mentioning a study by Hanewinckel and colleagues [99], which found a definite PNP prevalence of 5.5% in population screened with a detailed protocol including assessment of symptoms, neurological examination and nerve conduction studies. PNP appears to be more common in Western countries [97] and increases with age: it is present in approximately 13% of PNP subjects older than 80 years, making it a common cause of chronic pain in the elderly [91, 99, 100]. It also affects slightly more females than males [96, 97]. The prevalence of PNP increases with obesity, metabolic syndromes [92], and Diabetes Mellitus, which is a major acquired causes of PNP [99, 101]. Other acquired causes of PNP are vascular and blood problems, systemic autoimmune diseases, renal and hepatic disorders, nutritional deficiency (in particular vitamin B12 and B6), chemotherapy treatment and some forms of infections. Genetically-caused PNP are rare, some of which develop into mild forms of PNP in adulthood. A more severe inherited PNP is Charcot-Marie-Tooth disease, a sensory and motor PNP which appears in infancy or childhood [102].

ASSESSMENT AND DIAGNOSIS OF PERIPHERAL NEUROPATHY

Because of its different manifestations, e.g., altered sensation, pain, muscle weakness, atrophy and autonomic symptoms, PNP requires a comprehensive assessment to broaden the spectrum of symptoms analysis and improve its diagnosis [103]. Depending on the PNP type, an extensive assessment of PNP may include clinical, neurophysiological and neuropathological evaluations in order to characterize motor, sensory and autonomic alterations in detail [100].

Several clinical scales and questionnaires have been developed with the aim to investigate PNP signs and symptoms, ensuring reasonable correlations with neurophysiological tests and discriminatory characteristics [104].

Clinical scales and questionnaires can be divided in those investigating only symptoms, only signs or both. The Neuropathy Symptoms Score (NSS) and the Neuropathy Symptoms Profile (NSP), the latter a self-administered questionnaire, are among the main scales designed to quantify the symptoms of PNP. These scales have shown good correlation with neurophysiological tests and sural nerve structural changes [105, 106].

Major clinical scales using only signs include the Neuropathy Impairment Score for Lower Limbs (NIS-LL) and the Utah Early Neuropathy Scale (UENS). The NIS-LL is a comprehensive neurological examination focusing on the lower part of the body. It includes a series of tests on vibration thresholds and motor deficits. The UENS was developed to focus more on early signs [104, 106].

Finally, the main PNP scales that use both signs and symptoms are the Toronto Clinical Neuropathy Score (TCNS), the Michigan Neuropathy Screening Instrument (MNSI) and the Total Neuropathy Score (TCS), the latter being more specific to neuropathic impairments due to chemotherapy treatments. The TCNS can be used to measure changes in early neuropathic patients and has been validated against morphological criteria of sural nerve fiber density and nerve conduction velocities [107].

Taken all together, clinical scores allow detailed matching of neurological examinations of PNP at both levels of disability and functioning. However, they have several limitations, for example being complex and time consuming. Although administration is preferably done by neurologists, reproducibility depends on the training and background of the operator, and there is often a great variability among clinicians or among repetitions [104, 106, 107].

Nerve conduction studies (NCS) are electrophysiological examinations that record the electrical activity at various sites along the sensory and motor nerves [92]. In the evaluation of large fibers PNP, they can identify demyelinating and axonal features, as well as distribution, duration and course of PNP [100].

Large-fiber demyelinating PNP is characterized by slowing of nerve conduction velocity, and prolongation of terminal latency [92, 108]. In contrast, in large-fiber axonal PNP, the amplitude of responses is decreased or absent, with mild slowing of nerve conduction. In both axonal and demyelinating PNP, sensory nerve action potentials and sensory conduction velocities are usually reduced [108].

In order to define when outcomes are altered in the assessment of large-fiber PNP, various composite scores with all nerve conduction parameters have been developed. The main

composite score was proposed by Dick et al [109], who expressed nerve conduction parameters as centiles and normal deviates, based on normative population indices and adjusted by age, sex and BMI [110]. However, these quantitative approaches are global and based on large-fiber parameters, without defining impairments of small-diameter fibers. Consequently, the diagnosis of small-fiber neuropathy is not possible with this tool, but can be evaluated by other neurophysiological studies.

Thus, in order to quantify sensory function and investigate minor clinical signs of small fiber neuropathy, the use of Quantitative Sensory Testing (QST) has expanded in clinical routine. QST is a non-invasive instrument that measures thresholds of vibratory, thermal and painful stimuli. It can assess the entire sensory pathway, and can detect both hypoesthesia and hyperalgesia [111]. Because the sensory stimulus is reported subjectively, QST has the limitation of requiring high cooperation and attention from the patient, and may be influenced by psychological factors in the perception of sensory function [112]. Diagnostic sensitivity in small fiber neuropathy has been reported to range from 67% to 100% [113, 114]. However, its main limitation is its lack of specificity: some studies have shown a correlation between thermal and pain thresholds and the number of small nerve fibers in neuropathological examination [115-117], but other similar studies have shown opposite results [114, 118]. These findings suggested that QST should be used as an additional diagnostic test for small-fiber neuropathy, along with a comprehensive clinical assessment and neuropathological examination.

Finally, neuropathological evaluation measures the selective degeneration of small nerve fibers, which cannot be observed with routine neurophysiological tests. Quantification of small nerve fiber is performed via skin biopsy, a minimally invasive technique that has recently been expanded in clinical practice [119]. Skin biopsy quantitatively assesses somatic unmyelinated intraepidermal nerve fibers (IENF) in the human dermis and epidermis [120]. It allows detection of abnormalities in the cutaneous innervation and characterization of small-fiber neuropathy at different stages and of different types [103].

A 3-5 mm punch is usually performed on the distal part of the leg and, in addition, at the level of the proximal thigh (below the iliac spine), to investigate the length-dependent deterioration of small fibers [119, 120]. A major advantage of this method is the possibility to choose the skin biopsy site based on the neuropathic signs and symptoms experienced by the patient, or in regions where NCS cannot be performed (such as trunk or fingertips) [119].

The main guidelines on the use of skin biopsy in clinical practice and research provide standardized technical procedures and methodologies [120]. One of recommendation is the use of a non-specific antibody that binds to all axons, the PGP 9.5 antibody, a cytoplasmatic

marker that enables identification of small nerve fibers in the dermis and epidermis [121] (Fig. 3). Nerve fibers quantification is performed via bright-field immunohistochemistry or indirect immunofluorescence [122], both of which have been shown to have a high level of agreement and comparable validity [123]. Recent studies using these immunostaining techniques provided normative reference values that are now suitable for clinical use worldwide [124, 125]. These studies have shown that IENF density decreases with increasing age, and differs between males and females. The pathological decrease in small nerve fibers is confirmed when IENF density falls within the lower 5th percentile. The skin biopsy technique may also be useful to measure the progression of small fiber degeneration and study possible treatments [126].

A limitation of the use of skin biopsy for the investigation of PNP is its high variability with regard to the actual small nerve fiber quantification. The technique of IENF counting is time consuming and usually performed manually by 1-3 operators, leading to variable results and limited use in clinical setting [127]. There is still need to develop new, more standardized and automated counting techniques, with the aim to solve the lack of manual diagnostic accuracy and that can be easily applied in routine.

In conclusion, the diagnosis of PNP ca be challenging because its many clinical manifestations pose several difficulties in classifying all aspects of the disorder [128]. Accurate diagnosis of large-fiber neuropathy must precisely include a clinical examination of signs and symptoms of PNP and electrodiagnostic study findings (NCS), which are sensitive, specific and validated measures. On the other hand, small nerve fiber neuropathy cannot be diagnosed by NCS, because of their low resolution in detecting slow conduction velocities of small fibers. Therefore, to increase the reliability of small nerve fiber neuropathy diagnosis, at least two of the following tests must be altered: clinical signs and symptoms, abnormal QST results, and/or significant decrease in IENF density. Sensory symptoms alone should not be considered a reliable screening feature [129].

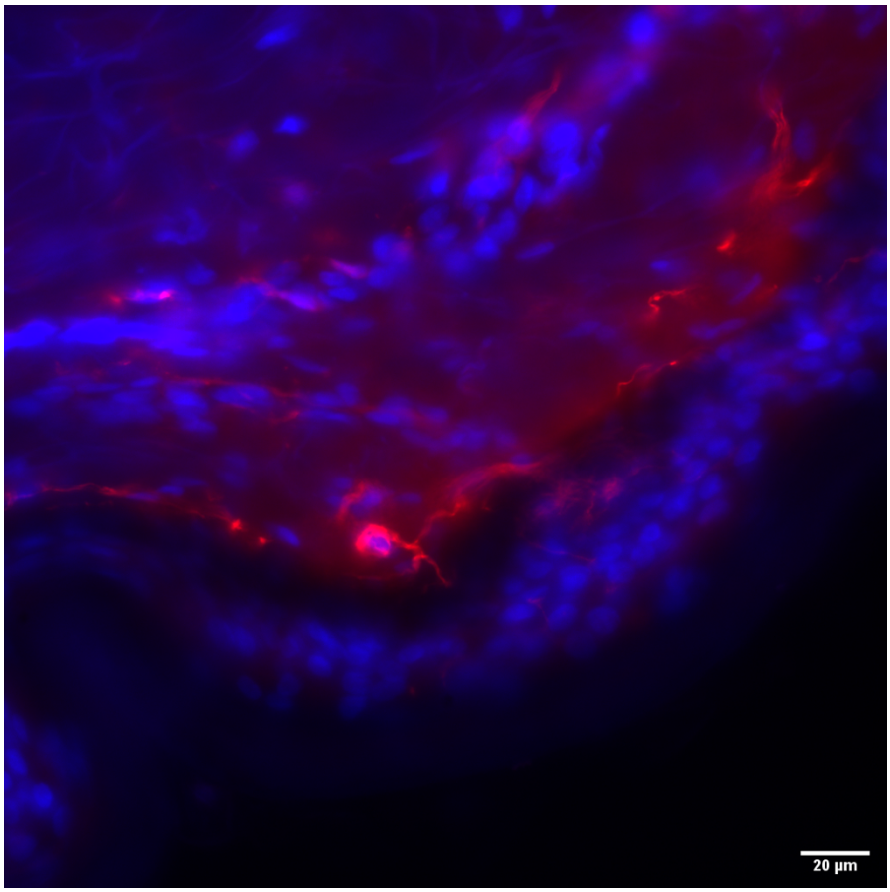

Figure 3. Intraepidermal small nerve fibers (in red) immune-stained with PGP 9.5 panaxonal antibody and cell nuclei of DAPI (in blue) in the human skin. Courtesy of Advanced Light Microscopy (ALM) platform, Instituto de Investigação e Inovação em Saúde da Universidade do Porto, i3s (PT).

PERIPHERAL NEUROPATHY IN PARKINSON'S DISEASE

The prevalence of PNP in PD varies widely, depending on the type of PNP investigated and the methods used. Large-fiber neuropathy in PD has a prevalence between 6% to 55%. A recent systematic review on PNP in PD addressed an estimated prevalence of large-fiber neuropathy of 16.3%, from a total of 17 studies and 1376 PD participants, confirming a higher incidence of PNP in PD than in the general population [90]. The same study showed identical gender ratio in participants with PD who developed PNP, and a higher mean age of participants with PNP compared with PD participants without PNP (69.4 and 66 years old, respectively).

Small-fiber neuropathy was investigated first by Novak [130] and then by Nolano [83] and colleagues, who demonstrated reduced small fiber density in PD subjects. Based on IENF density, the reported prevalence of small-fiber neuropathy ranged from 37% to 91%,

whereas the pooled estimated prevalence was 56.9%, reported in 3 studies with a total of 72 participants with PD [90].

Two main hypotheses regarding the presence of PNP in PD have recently been proposed. One group of studies has focused on the link between PNP and L-dopa exposure, as PNP has been shown to become more frequent as the drug dose increases (and thus presumably in the late stages of the disease). On the other hand, PNP was observed in the early stages of PD, wondering whether peripheral nervous involvement could be considered an intrinsic part of PD degeneration [131].

With regard to the first hypothesis, the association between PNP and long-term L-dopa treatments has been investigated in several large studies. Long-term L-dopa exposure has been linked to PNP because chronic L-dopa intake leads to a sequence of events which alters the peripheral nerve homeostasis, and, consequently, causes peripheral nerve damage. At the neuropathological level, neuropathological changes could be related to exposure to toxic metabolites (such as homocysteine, Hcy, formed via conversion of L-dopa to dopamine) and to decreased levels of vitamin B (particularly VB12), resulting from Hcy accumulation [89, 131].

Several large studies have confirmed elevated Hcy levels in patients with PD, and a higher prevalence of PNP in patients with long L-dopa use [132, 133]. A study by Rabajally and colleagues [134] identified and association between PNP and VB12 deficiency, long-term L-dopa doses, and disease duration in 38% of PD participants. A multicenter study stratified PD participants with long L-dopa exposure and found that 19.4% of participants with long L-dopa exposure had PNP, whereas in the PD groups with short L-dopa exposure, only the 6.8% had PNP [135]. The PD-PNP group with long L-dopa exposure also showed high Hcy and reduced VB12 levels. Several other descriptions of PNP related to chronic L-dopa exposure have been reported [136, 137].

The second hypothesis investigates PNP as intrinsic aspect of PD characteristics. Mild subclinical signs and symptoms of PNP have been observed in PD patients, regardless of L-dopa exposure and therapy, often in the early stage of disease [131]. Therefore, peripheral involvement has been considered as part of the neuro-degeneration from the earliest stages of the disease. Studies in favor of this hypothesis have shown the presence of α-synuclein deposition in small nerve fibers (cutaneous fibers) via skin biopsies, but not in patients with multiple system atrophy or essential tremor [138, 139]. Detection of α-synuclein deposition in dermal nerve fibers may be a useful diagnostic and investigative tool of PD onset and progression.

Finally, an important aspect of the presence of PNP is its functional impact on mobility in PD. It has been already confirmed that individuals with PNP are 15 times more likely to fall

than healthy controls [140]. Falls are reported to be a dangerous health problem that consequently leads to reduced independence and quality of life, and higher mortality rates [141]. Patients with PNP have the tendency to show more impaired and variable spatial-temporal parameters during walking (such as slower gait speed and lower stride length) and postural instability, which could be the result of motor weakness, proprioceptive deficits and impaired sensorimotor functions [142, 143]. The association between PNP and PD in motor performance is still poorly investigated. A study by Beaulieu and colleagues [144] confirmed that the presence of PNP in PD was significantly associated with impaired gait performance. However, gait analysis was performed in a small 8-m walkway which did not gather all motor functions. The functional impact of PNP in PD has never been studied with the use of wearable health technology, which may collect more complete information about mobility of this particular subset of PD patients affected by PNP. A more detailed analysis of gait and balance characteristics of PD-PNP patients is still lacking.

CHAPTER 2.
OBJECTIVES

PD is a complex and multifaceted phenomenon whose major symptoms progressively result in increased disability and reduced quality of life. Precise assessment and characterization of all PD hallmarks are critical to monitor disease progression and adapt ongoing treatments. The use of wearable health-technology in clinical research has provided the ability to continuously monitor PD motor symptoms and fluctuations, but it has also brought attention to the differences between assessments in supervised laboratory-settings and unsupervised environments, such as at home. It is still unclear whether supervised and unsupervised assessments are as sensitive when used to identify motor fluctuations and everyday-performance in PD.
For this reason, in order to broaden information about PD daily-life performance and improve clinical care with targeted and individualized interventions, the first aim of the research was to compare PD mobility in the laboratory and at home with wearables, and in particular to investigate which supervised gait tests could most accurately represent the performance of PD patients at home, also in association with medication states and clinical scores.

The second aim of our research was to investigate an aspect of PD disability by studying the functional impact of PNP on gait and balance, using wearable health-technology. Due to its wide spectrum of clinical manifestations, PNP can significantly worsen the overall functional mobility of patients with PD, but this functional aspect has never been widely investigated. We aimed to first elaborate a systematic review to examine the most relevant and clinically useful methodologies to characterize PD- and PNP- associated gait and balance deficits, as well as the main associated motor parameters. In addition, we aimed to propose future indications for the assessment of PD patients with PNP (PD-PNP) with this technology. Subsequently, we aimed to use the proposed protocol with wearable sensors to investigate the effect of PNP on gait and balance on a consecutive cohort of PD patients. This study was divided into two parts. The first goal was to perform a comprehensive assessment to characterize PNP and PNP types (large- and small-fiber neuropathy) in PD. Second, we aimed to assess gait and balance at different medication states, to determine whether PNP, and PNP types, were a contributing factor to impaired motor deficits.

As a final part of the project, the major limitations of the manual quantification of small nerve fibers in skin biopsy, namely being time-consuming and the high inter-operator variability, were also confirmed during the evaluation of small-fiber neuropathy in our PD cohort. In

order to overcome these limitations of manual counting, we aimed to develop a new, less-operator dependent method for small nerve fibers counting, to be applied both in research and clinical practice.

CHAPTERS 3.

COMPARISON OF LABORATORY AND DAILY-LIFE GAIT SPEED ASSESSMENT DURING ON AND OFF STATES IN PARKINSON'S DISEASE

This Chapter was adapted from the published work:

Corrà MF, Atrsaei A, Sardoreira A, Hansen C, Aminian K, Correia M, Vila-Chã N, Maetzler W, Maia LF. Comparison of Laboratory and Daily-Life Gait Speed Assessment during ON and OFF States in Parkinson's Disease. Sensors (Basel). 2021 Jun 9;21(12):3974. doi: 10.3390/s21123974.
Full Open Access publication

ABSTRACT

Accurate assessment of Parkinson's disease (PD) ON and OFF states in the usual environment is essential for tailoring optimal treatments. Wearables facilitate measurements of gait in novel and unsupervised environments; however, differences between unsupervised and in-laboratory measures have been reported in PD. We aimed to investigate whether unsupervised gait speed discriminates medication states and which supervised tests most accurately represent home performance. In-lab gait speeds from different gait tasks were compared to home speeds of 27 PD patients at ON and OFF states using inertial sensors. Daily gait speed distribution was expressed in percentiles and walking bout (WB) length. Gait speeds differentiated ON and OFF states in the lab and the home. When comparing lab with home performance, ON assessments in the lab showed moderate-to-high correlations with faster gait speeds in unsupervised environment ($r = 0.69$; $p < 0.001$), associated with long WB. OFF gait assessments in the lab showed moderate correlation values with slow gait speeds during OFF state at home ($r = 0.56$; $p = 0.004$), associated with short WB. In-lab and daily assessments of gait speed with wearables capture additional integrative aspects of PD, reflecting different aspects of mobility. Unsupervised assessment using wearables adds complementary information to the clinical assessment of motor fluctuations in PD.

Keywords: remote patient monitoring; medication states; Parkinson's disease; lab vs. home; wearable sensors; human gait; gait speed

INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder characterized by impairment of mobility and gait, with severe consequences on quality of life. Motor symptoms and gait impairment are mainly caused by loss of dopaminergic neurons in the substantia nigra, decreasing dopamine levels in the brain [1]. Thus, current treatments of PD focus on increasing dopamine delivery: among the main dopaminergic medications, levodopa is considered the gold standard therapy [2]. Up to 50% of PD patients within two years of levodopa therapy may begin to experience mild motor fluctuations [3]. Motor fluctuations represent alternations of periods of good dopaminergic effect, with adequate control of movements (the perception of this state by the affected patient is called "ON state") to others of poor control and significant worsening of motor symptoms (comparably, this perception by the affected patient is called "OFF state") [4].

Reduction of motor fluctuations is an important indicator to evaluate the effectiveness of pharmacological interventions. The most popular tool to quantitatively assess motor fluctuations is the motor part of the Unified Parkinson's Disease Rating Scale (UPDRS, and the Movement Disorder Society-revised version thereof), a validated clinical rating scale of PD symptoms that includes both historical information and clinical examination for ON and OFF states [5]. However, clinical scores do not necessarily provide representative data on the patient's daily performance at home. An alternative method of tracking motor fluctuations is to ask patients to fill a diary differentiating various symptoms during the day, and to rate and define their current status of being at ON state or at a decreased medication effect by self-perception. This method has several limitations, including recall bias, reduced compliance, and the need to be accurately compiled to have valid and interpretable data [6]. In addition, these methods do not involve quantitative measures of movements. Monitoring objective gait parameters to track motor fluctuations may compensate these subjective limitations, and allow more sensitivity, accuracy and reproducibility [7].

More recently, wearable health technologies have been developed with the possibility to investigate PD symptoms at a new level of granularity and in novel environments that have previously not been covered by clinical evaluations [8]. In particular, inertial measurement units (IMUs) are electronic devices worn on the body that can detect movements and successfully estimate spatial-temporal parameters, using a combination of accelerometers, gyroscopes and sometimes magnetometers. Thanks to the reduced size and costs of their components, they are easy to wear and low-cost tools for movement analysis [8]. Compared to more complex equipment such as 3D optical motion capture systems, IMUs are less time consuming and do not require specific expertise to use. In addition, complex tools are used only in clinical settings due to their high cost and complexity of technology, and do not often represent the full gait complexity [9]. The use of inertial measurement units (IMUs) indeed makes it now possible to investigate motor features such as gait and motor performance in

unsupervised conditions such as the domestic environment. This may enable a passive collection of clinically important information, such as durations of medication ON and OFF states, in natural environments of individuals [10–12]. Among the parameters extracted from IMUs, gait speed was shown to be one of the most reliable predictors of mobility [13], risk of falling [14,15], and loss of independence [16], as well as a powerful indicator of changes in performances over time [17]. Gait speed is a critical measure of gait function for different pathologies [13,18,19]. Stratification of gait activity in the home environment in walking bouts (WBs) of different lengths seems to provide additional useful insight into mobility performance [20].

It has been observed that gait speed can significantly differ when analyzed at home or in the laboratory [13,19,21–24]. Carcreff et al. [13] showed lower gait speed values in daily life compared to lab in a group of children with cerebral palsy. In this study, children were asked to walk barefoot during the lab assessment, which may generate great differences when comparing both assessments. Two other studies [19,23] have compared supervised and unsupervised gait speeds in the elderly. A weak association between daily-life- and lab obtained gait speed was found by Takayanagi et al. [19], with average daily gait speed being significantly lower than lab speed. De La Camara et al. [23] showed an association with speed and physical, mental and cognitive health outcomes, and highlighted that clinically obtained gait speed can underestimate or overestimate habitual gait speed. All of these studies used IMUs to detect mobility, but they all presented limitations regarding the type of gait tasks used for the lab assessment. For example, distance walked in the lab was between 2.44 and 10 m, which can be considered too short to be compared with daily gait speed. Even in PD, it has been shown that supervised instruments to measure motor symptoms do not strongly reflect daily-living activity [21]. For example, no significant correlation was observed between lab and home gait parameters in a study by Toosizadeh et al. [22], but the small sample size and methodological differences (one single sensor on the sternum) between lab and home assessments of the study may have affected the accuracy of the results. Therefore, it is still unclear whether supervised and unsupervised assessments are as sensitive when used to identify motor fluctuations in PD. Precise information on the degree of association between supervised and unsupervised assessments of motor fluctuations is still lacking. Improving the understanding of gait disabilities with additional information about daily-life performances from IMUs is essential to enhance clinical care, design personalized interventions and overcome limitations concerning questionnaires and self-reported diaries.

For these reasons, in the present study, we compared gait speed from supervised (in the laboratory) and unsupervised (at home and daily-life conditions) assessments to determine the degree of association of the different medication states in PD patients. In particular, we

tested whether gait speed in unsupervised environments discriminates ON and OFF states, and investigated which supervised tests most accurately represent home performance during both medication states, also in relation to clinical scores.

MATERIALS AND METHODS

Patients

PD patients diagnosed by a movement disorder specialist based on the UK Brain Bank criteria [25] were recruited. The following exclusion criteria were applied:

- Older than 90 years of age;
- Dementia;
- Any relevant gait-impairing health issue other than PD;
- Unable to walk a distance of 20 m;
- Not taking anti-parkinsonian medications in the past month;
- Difference of less than 2 points between ON and OFF in the UPDRS-III, in order to consider the minimum clinically significant difference between states [26,27].

Demographic data and information on medication intake were collected [28]. The main characteristics of the population are presented in the results section. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Centro Hospitalar Universitário do Porto (CHUP) with identification code 2018.087(076-DEFI/076-CES).

Data Collection

During the first day of assessment, participants were first evaluated with UPDRS part III (a medical professional evaluates actual motor performance) in their OFF state in the morning, at least 12 h after the last dose of dopaminergic medication intake. They were then equipped with two synchronized RehaGait IMUs (Hasomed GmbH, Magdeburg, Germany), each containing a tri-axial gyroscope and tri-axial accelerometer (Figure 1). These sensors were located on both feet, recording data with a sampling frequency of 100 Hz. Participants were asked to perform the following gait tasks: a 20-m straight walking test at normal pace and at fast pace; and a circular walking task, which is walking at normal pace three times around a circular carpet of 1.2 m diameter, at both left and right directions (Figure 1). Then, participants were asked to take their usual first dose of dopaminergic medication, and the same assessment (UPDRS scores and gait assessment) was performed between 1 and 2 h after intake of medication.

During the second day of assessment, participants wore a Physilog® 5 IMU (Gait Up, Lausanne, Switzerland) on the right foot between 9:00 and 9:30 a.m. (Figure 1). The device comprises a triaxial accelerometer and gyroscope with a sampling frequency of 128 Hz. For the following 12 h (during a weekday), participants were then asked to keep wearing the IMUs and perform usual daily activities in their domestic environment, including their usual outdoor activities. For convenience, the terms 'home' or 'domestic environment' will include outdoor activities during the day of the assessment.

Both inertial sensors used in the study showed the same technical characteristics and measure the same type of data, guaranteeing no differences in terms of results and set-ups (Figure 1). In addition, participants were asked to fill in a diary over the day to report the times of dopaminergic medication intake. To ensure a precise documentation of time and quantity of dopaminergic medication intake, caregivers were also instructed to monitor the diary record.

Figure 1. (**A**) Lab setting for the supervised assessment: 20 m walking and circular walking tasks. (**B**). Sensor positioning for lab assessment. (**C**). Sensor positioning for unsupervised assessment.

Data Processing and Extracted Parameters

Raw data were processed using Matlab R2020b (MathWorks, Nantick, MA, USA). To analyze gait speeds, the raw data of the IMU from the right foot 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 algorithm to obtain

gait speed [29]. This algorithm had been validated in a previous study on PD patients and achieved an accuracy (±precision) of 2.8 (±2.4) cm/s [30].

From the lab assessment, gait speed was calculated for all walking tests (20-m walk test at normal and fast pace, and the circular walking test). Two strides at the beginning and end of the tests, respectively, were excluded to obtain steady-state gait speed values. As described in the reference [29], the raw accelerometer and gyroscope data were processed to first detect the gait events. The acceleration of the movement was integrated to obtain the velocity of the foot. During the motionless periods, the zero-velocity update approach was applied to overcome the drift problem. Gait speed was then calculated from the drift-free velocity. Details of the procedure are provided in [29]. An example of extracted data is shown in Figure 2.

From the home assessment, due to the complex context of the unsupervised setting, and the vast distribution of gait speed [24], two approaches were employed. In the first approach, gait speed was obtained from each stride. In the second approach, mean gait speed per walking bout (WB) was calculated. WBs were determined as described earlier [31], and then divided into short (15–30 s), medium (>30–60 s) and long WBs (>60 s). WBs shorter than 15 s were not included to avoid any influence on the accuracy of the used algorithm. Gait cycles having less than 0.2 m/s of gait speed were not considered, as they can be assumed to be static periods. Gait bouts were then allocated to respective ON and not-ON states: ON state was arbitrarily defined as 60–180 min after dopaminergic medication intake (based on the dopaminergic intake time in the diary). The period between 30 min before and 30 min after dopaminergic medication intake was defined as not-ON state, describing the condition in which no optimal drug effect is to be assumed [32–34].

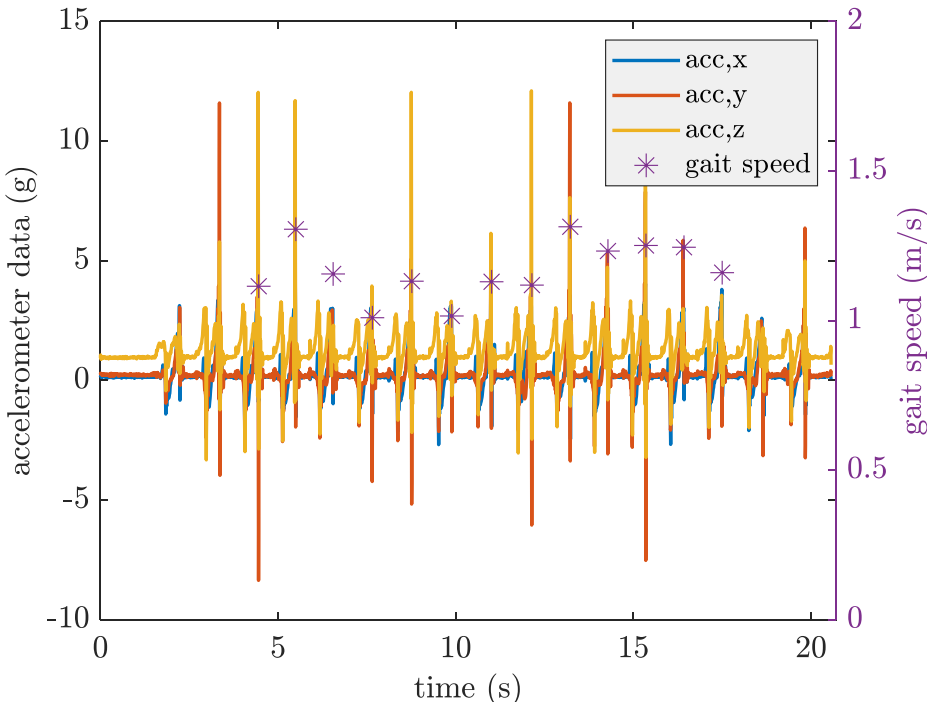

Figure 2. Example of gait speed extraction from a PD patient during straight walking at fast pace. Gait speed is given for each gait cycle of the right foot.

Statistical Analysis

From the lab data, mean gait speeds of each trial were extracted. From home-based data, the 25th, 50th, 75th, 90th percentiles and maximum values of gait speeds were calculated from all gait cycles. The use of different percentiles was based on previous studies showing a heterogeneous distribution of daily-life gait speed in the elderly, resulting in relevant correlations with higher percentiles with capacity in the lab [35].

From the WB approach, we calculated the mean gait speed within each WB type, and the corresponding 25th, 50th and maximum values of each WBs type were considered for both ON and not-ON medication states [35].

Normality of data was checked with Shapiro–Wilk test. To compare different medication states in the lab (ON, OFF) and home environment (ON, not-ON), a paired comparison (t-test for parametric data; Wilcoxon signed rank test for non-parametric data) was used, and p values < 0.05 were considered significant. I

In order to compare PD patients' gait speed at home and in the lab at both medication states, respectively, and in relation to clinical scores, we performed a correlation analysis (Pearson correlation for parametric data; Spearman correlation for non-parametric data). A correlation coefficient of less than 0.5 was considered as low, between 0.5 and 0.7 as moderate and above 0.7 as high [36]. In addition, to measure the proportion of the variance of home assessment that is predictable from the lab assessments, the coefficient of

determination (R^2) was calculated, applying data transformation for non-normally distributed variables. All statistical analysis was performed using IBM® SPSS 25 package.

RESULTS

Demographic and Clinical Characteristics

A total of 39 PD patients were initially recruited. Out of this group, a total of 27 patients (40.7% female) met the inclusion criteria and performed the entire study protocol. Included participants did not significantly differ in demographic and clinical characteristics from those not included (Supplementary Table S1). Median age of the included participants was 69 years and the median disease duration was six years. Seventeen PD patients (63%) were early stage (Hoehn and Yahr stage 2 during medication OFF state), and 10 patients (37%) at mild-to-moderate stage (Hoehn and Yahr stage 2.5–3). Demographic and clinical details are provided in Table 1.

Table 1. Demographics and clinical characteristics of PD patients.

Variables	Value (IQR)
Male: Female	16:11
Age (years)	69 (64–73)
Disease duration (years)	6 (3–9)
Disease onset (years)	64 (57–69)
Hoehn and Yahr stage (0–5)	ON: 2 OFF: 2
UPDRS I (0–16)	2 (1:4)
UPDRS II (0–52)	7 (3:10)
UPDRS III (0–108)	ON: 12 (8:20) OFF: 22 (15:31)
UPDRS IV (0–23)	2 (0:3)
Total LED (mg)	580 (400–770)

Note: Results are expressed in median and interquartile range (IQR). LED: Levodopa equivalent dose; UPDRS: Unified Parkinson's Disease Rating Scale.

Gait Speed

Comparison of Gait Speeds between Respective ON and OFF/not-ON States

Relative gait speeds in the lab and at home are shown in Figure 3. In the lab, straight walking at normal pace had a median value of 1.01 m/s during the medication ON state, and 0.97 m/s during the medication OFF state ($p = 0.004$). During circular walking it reached 0.69 m/s during the ON, and 0.58 m/s during the OFF state ($p < 0.001$). Only two PD patients

were able to perform the straight walking at fast pace assessment during the medication OFF state; therefore, this task was not included in the analysis.

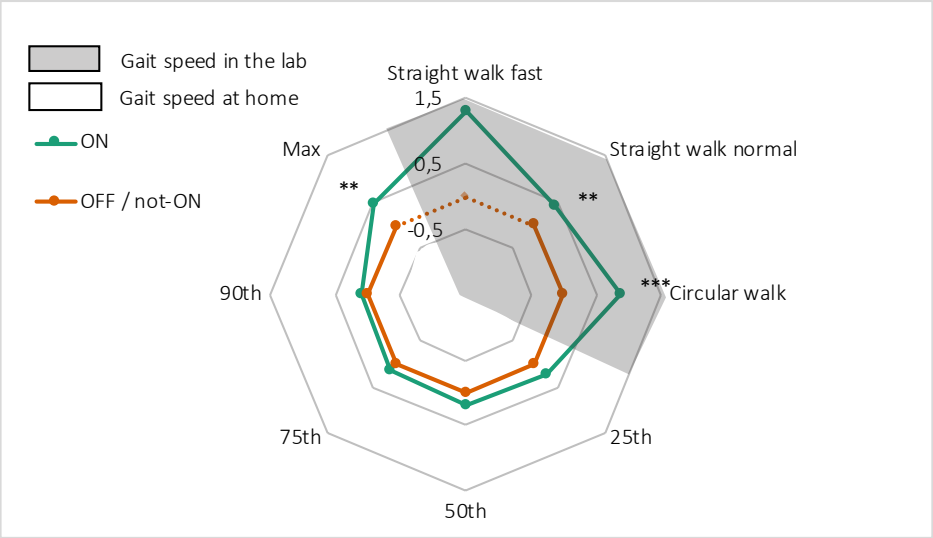

Figure 3. Radar plot illustrating gait speeds of 27 PD patients during lab tests (in grey) and in their domestic environment (white, presented by different percentiles). Gait speeds during medication OFF in the lab and not-ON in the domestic environment are presented as 0 (orange line), and gait speeds during medication ON are presented as deviation from 0 (green line). Straight walking test at fast pace was not performed during OFF medication state (dotted orange line). ** $p < 0.01$, *** $p < 0.001$.

In the domestic environment, the median gait speed was 0.83 m/s during the medication ON state and 0.77 m/s during the medication not-ON state (p = 0.302). A significant difference was found between ON and not-ON medication states for the maximum gait speed (ON = 1.48 m/s; not-ON = 1.36 m/s; p = 0.009), but not for the other percentiles (Figure 3).

Comparison of Gait Speeds between Lab and Home Environment during Medication ON State

During the medication ON states, low correlations were found when comparing gait speeds obtained from the normal walking tasks in the lab with the maximum values of home gait speed (r = 0.46; p = 0.02). Moderate correlations of gait speeds were observed between the fast walking task in the lab and the 90th percentile (r = 0.64; p < 0.001) and maximum values (r = 0.69; p < 0.001) of the home-derived data. Similar results were found when comparing the circular walking task in the lab with the 90th percentile (r = 0.53; p = 0.004) and maximum values (r = 0.61; p = 0.001). In general, the degrees of correlation between

lab and home gait speeds increased with higher percentiles of gait speed in the unsupervised environment. This was also reflected by the R^2 values (Table 2; Figure S1).

Comparison of Gait Speeds between Lab and Home Environment during Medication OFF/not-ON State

During the medication OFF/not-ON states, moderate correlations were found when comparing gait speeds obtained from the normal walking tasks in the lab with the 25th percentile of home gait speed (r = 0.56; p = 0.004). Similar results were also found when comparing gait speed of the circular walking task in the lab with the 25th percentile of gait speed as obtained from the home data (r = 0.55; p = 0.004). This was also reflected by the R^2 values (Table 2; Figure S1).

Table 2. Correlation of ON and OFF/not-ON state between the lab and the domestic environment.

	ON								
	Straight Walking Fast Pace			**Straight Walking Normal Pace**			**Circular Walking**		
	Comparison	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**
	***p*-Value**	***r***		***p*-Value**	***r***		***p*-Value**	***r***	
25th	<0.001	0.45 *	20	<0.001	0.38 *	14	0.715	0.36	13
50th	<0.001	0.54 **	29	0.003	0.40 *	16	<0.001	0.49 **	25
75th	<0.001	0.60 **	37	0.495	0.40 *	16	<0.001	0.53 **	28
90th	<0.001	0.64 ***	41	0.132	0.40 *	16	<0.001	0.53 **	28
Max	<0.001	0.69 ***	30	<0.001	0.46 *	15	<0.001	0.61 **	39
	OFF/not-ON								
	Straight Walking Fast Pace			**Straight Walking Normal Pace**			**Circular Walking**		
	Comparison	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**
	***p*-Value**	***r***		***p*-Value**	***r***		***p*-Value**	***r***	
25th				<0.001	0.56 **	33	0.038	0.55 **	31
50th	Straight walking test at fast pace was			0.009	0.42 *	18	<0.001	0.39 *	16
75th	not performed during OFF medication			0.893	0.36	14	<0.001	0.38 *	15
90th	state.			0.030	0.34	12	<0.001	0.41 *	17
Max				<0.001	0.15	2	<0.001	0.37	14

Paired comparisons (*p*-value), degrees of correlation (*r*) and coefficients of determination (R^2) between lab tests and most relevant percentiles of gait speed in the domestic environment during ON and OFF/not-ON medication state. Correlation asterisks represent the following *p*-values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Comparison of Gait Speeds between Lab and Home Environment during Opposite Medication States

Low correlations were found when comparing gait speeds obtained from the normal walking task in the lab at ON state with the maximum values of home gait speed at not-ON ($r = 0.26$; $p = 0.209$). Moderate correlations of gait speeds were observed between the fast walking task in the lab at ON and the maximum values of the home-derived data at not-ON ($r = 0.47$; $p = 0.015$). Similar results were found when comparing the circular walking task in the lab at ON with maximum value of home gait speed at not-ON ($r = 0.41$; $p = 0.035$) (Supplementary Table S2). The same lower percentiles were observed between measurements at OFF in the lab and ON in domestic environment: gait speed from the normal walking tasks at OFF and the 25^{th} percentile from home at ON ($r = 0.38$; $p = 0.063$); circular walking task at OFF and the 25^{th} percentile from home at ON ($r = 0.43$; $p = 0.031$).

Comparison of Gait Speeds in the Home Environment, Stratified by Different Bout Lengths

In the home environment, WBs of different lengths most probably reflect different purposes of walking, such as doing the housework (short WBs) and taking a walk (long WBs) [37]. We found no significant differences in the number of WBs between ON and not-ON medication states (Supplementary Table S3). When comparing gait speeds between ON and not-ON states, stratified by different WB lengths, we found significant differences between ON and not-ON medication states only at high gait speeds. Moreover, this was only observed in short and medium WBs (short WBs: $p = 0.026$; medium WBs: $p = 0.008$). Long WBs did not add relevant information (Figure 4).

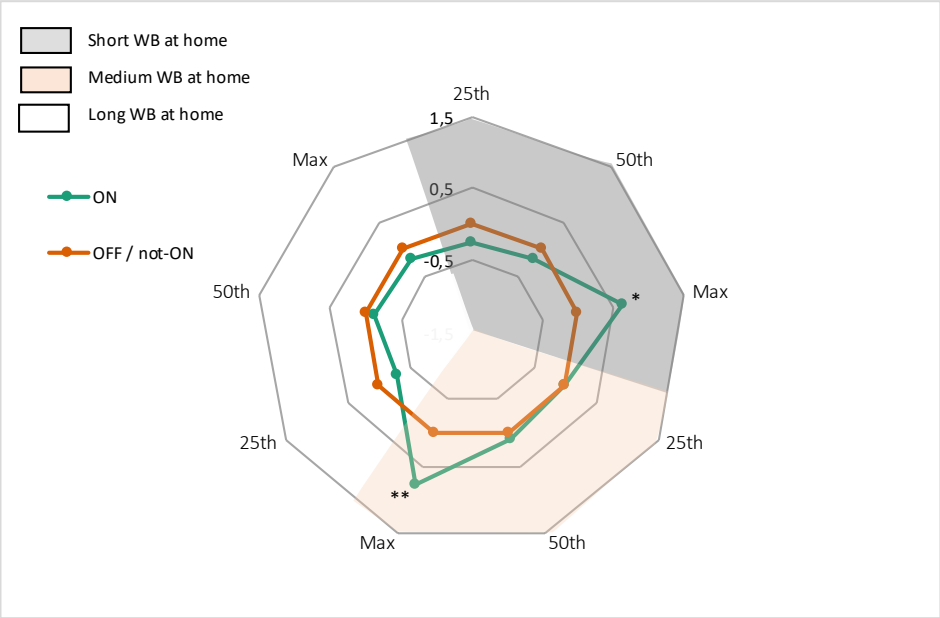

Figure 4. Radar plot illustrating gait speeds of 27 PD patients in their domestic environment, broken down into different WB. Gait speeds during medication not-ON are set at 0 (orange line), gait speeds during medication ON are presented as deviation from 0 (green line). * $p < 0.05$, ** $p < 0.01$.

We then compared gait speeds from the lab with those obtained from the domestic environment during ON state, stratified by WB lengths. Degrees of correlation were highest between the fast walking task in the lab and the maximum gait speed during long WBs (r = 0.63; p < 0.001) and the circular walking task in the lab and the 50th percentile of gait speed during short WBs (r = 0.72; p < 0.001) (Table 3; Figure S2).

Consistently, when comparing gait speed between the lab and the domestic environment during OFF/not-ON state, stratified by WB lengths, degrees of correlation were highest between the normal walking task in the lab and the 25th percentile of gait speed during short WBs (r = 0.57; p = 0.004), and the circular walking task in the lab and the 25th percentile of gait speed during short WBs (r = 0.58; p = 0.003) (Table 3; Figure S2).

Table 3. Correlation of ON and OFF/not-ON medication states between the lab and the domestic environment according to WB.

		ON								
		Straight Walking Fast Pace			**Straight Walking Normal Pace**			**Circular Walking**		
		Comparison	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**
		***p* Value**	***r***		***p* Value**	***r***		***p* Value**	***r***	
Short	25th	<0.001	0.33 *	11	<0.001	0.31	10	<0.001	0.66 ***	44
	50th	<0.001	0.49 *	28	<0.001	0.32	10	<0.001	0.72 ***	52
	Max	<0.001	0.39 *	16	0.09	0.24	6	<0.001	0.49 **	24
Medium	25th	<0.001	0.17	3	<0.001	0.09	1	<0.001	0.51 **	26
	50th	<0.001	0.16	8	<0.001	0.05	0.3	0.532	0.39 *	16

	Max	<0.001	0.51 **	27	0.046	0.27	8	<0.001	0.40 *	17
	25th	<0.001	0.28	8	<0.001	0.24	6	0.04	0.45 *	21
Long	50th	<0.001	0.61 **	37	0.005	0.42*	18	<0.001	0.5 **	28
	Max	<0.001	0.63 **	41	0.649	0.29	9	<0.001	0.38 *	14

OFF

		Straight walking fast pace			**Straight walking normal pace**			**Circular walking**		
		Comparison	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**	**Comparison**	**Correlation**	**R^2 (%)**
		***p* Value**	***r***		***p* Value**	***r***		***p* Value**	***r***	
	25th				<0.001	0.57 **	18	0.037	0.58 **	28
Short	50th				<0.001	0.39 *	16	0.617	0.44 **	19
	Max				0.02	0	0	<0.001	0.16	3
	25th	Straight walking test at fast pace			<0.001	0.49 *	25	0.333	0.55 **	31
Medium	50th	was not performed during OFF			<0.001	0.51 *	27	0.006	0.50 *	26
	Max	medication state.			<0.001	0.51 *	28	<0.001	0.55 *	28
	25th				0.028	0.38	15	<0.001	0.17	3
Long	50th				0.167	0.42	18	<0.001	0.18	4
	Max				0.322	0.2	4	<0.001	0.05	0

Paired comparisons (*p*-value), degrees of correlation (*r*) and coefficients of determination (R^2) between lab tests and WB of gait speed in the domestic environment during ON and OFF/not-ON medication state. Correlation asterisks represent the following *p*-values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Comparison of Home-Collected Gait Speeds with UPDRS-III Scores

At ON state, there were no significant correlations between the UPDRS-III ON scores and the home-collected gait speed percentiles. Only item 30, assessing gait, significantly correlated with the 90th percentile of home-collected gait speed (r = -0.61; p = 0.001).

At not-ON state, there were no significant correlations between the UPDRS-III OFF scores and the home-collected gait speed percentiles at not-OFF. Only item 30 moderately correlated with the 25th percentile of home-collected gait speed (r = -0.44; p = 0.028).

DISCUSSION

This exploratory cross-sectional study with PD patients during medication ON and OFF/not-ON states investigates gait speeds obtained from the lab and from a home assessment.

Firstly, gait speed was the only objective parameter considered in this analysis. Due to its combination of temporal and spatial gait characteristics, it is the most reliable predictor of mobility, and a valid and easy-to-administer measure of walking, which can reliably be estimated using IMUs [13].

Secondly, we decided to use different gait tasks in the lab and different set-ups in order to gather inertial signals similar to those that would be obtained in the daily life of PD patients.

Other studies have only compared daily-life gait speed with tasks of short distances at normal pace, making this comparison less reliable [13,19,22,23]. We confirmed the importance of using both lab and home assessments to add considerable explanatory value to the understanding of PD motor function.

We found relevant differences in maximum gait speeds in the home environment between medication ON and not-ON states (Figure 1). When analyzing the best performance at home (i.e., the maximum values of gait speed at home) we were able to discriminate medication states. Moreover, when we analyzed gait speeds stratified by WB lengths, we found that maximum values of gait speed during short and medium WBs provided more informative to discriminate ON and not-ON states in PD. This finding suggests that maximum values of gait speed at home better represent the maximum capacity in the lab rather than normal daily-life performance. This may help clinicians to have a more precise estimation of the patient's capacity when measuring mobility in unsupervised conditions. Considering extreme values of gait speed may be more informative when measuring mobility and the patient's motor status. Furthermore, shorter walking bouts provide more discriminative information compared to longer walking bouts as short walking bouts might be accompanied by other cognitive or motor tasks. Therefore, we recommend considering these parameters as useful indicators when evaluating PD treatment's effect in daily-life environments [22,24,37]. In unsupervised settings, the segmentation of WBs by length (short and medium WBs) provided additional information on individual patient ON/not-ON state.

We report, to the best of our knowledge, for the first time, that specific percentiles of gait speed and WB lengths may help in differentiating and monitoring ON and not-ON states in PD. Previous studies aimed to differentiate PD states in the home environment using other parameters rather than gait [38], or through the development of algorithms and machine learning approaches for quantifying specific PD motor symptoms, such as tremor, bradykinesia and dyskinesia [39–42]. Our study adds to such studies by including unsupervised gait speed performance as a relevant parameter to accurately monitor PD patients with mobile health technology, ultimately aiming at personalized adjustments in PD therapy.

We found that gait speed as assessed in medication ON state in the lab reflects (i) gait speeds obtained in the home during ON are better than during not-ON states, (ii) faster speeds correlated higher than slower speeds, and (iii) high correlations were mainly obtained in the long WBs. In more detail, we found the strongest association of fast pace in the lab with the highest gait speed percentiles ($r = 0.69$; $p < 0.001$) and long WBs (0.63; $p = 0.002$) of daily gait speed. This supports the concept that maximum capacity in the lab

can efficiently reflect the best performance in daily life, and that assessments of capacity are possible in both lab and home environments.

In contrast, daily gait speed showed only low correlation with walking at preferred pace in the lab (r = 0.46). This lab gait task may thus not reliably reflect the complexity of mobility at ON state in everyday life, probably because asking to walk at 'considered normal' speed may lead to different interpretations, and cause a less homogeneous speed. Therefore, assessing fast speed in the lab may give more information on motor functions during daily-life activities. These discrepancies are also in line with previous studies: no significant correlation was found between walking at normal pace in the lab and home assessment [22], and lab-based gait assessment explained less than one third of the daily-living activity in PD [21]. This may be related to an increased awareness when performing tasks in a supervised environment or because of limited ecological validity when performing isolated movements [21,22,24]. Therefore, assessing fast speed in the lab may give more information on motor functions during daily-life activities.

We also observed that circular walking in the lab can moderately represent a patient's everyday gait speed at home (r = 0.61). This association was pronounced in short WBs, which may reflect more complex and demanding gait situations in daily life, such as specific activities including walking and acting with the hands simultaneously [37,43]. Evidence from literature supports this hypothesis: gait features obtained in unsupervised conditions were closer to gait features obtained in the lab during dual-tasking, than when only walking [44–47].

In contrast, gait speed as assessed in medication OFF state in the lab reflects (i) gait speeds obtained in the home during not-ON are better than during ON states, (ii) slower speeds correlated higher than faster speeds, and (iii) high correlations were mainly obtained in the short WBs. In more detail, gait speed obtained during OFF state in the lab seems to best reflect how PD patients perform at home when below their usual performance, confirmed by the association of home-collected gait speed of lower percentiles (25th) and short WBs with all the lab tests (r = 0.56; p = 0.004). This is contrary to the results observed during respective ON states, where the higher was gait speed in the domestic environment, the higher was the correlation value with fast gait speed in the lab. Nevertheless, for this specific dataset, less information was obtained during not-ON state.

Taken together, these findings show that, during medication ON states, respectively, the fast speed gait value obtained in the lab can nicely inform about how PD patients perform their fast walking activities in their usual environment, and, similarly, circular walking can provide information about how PD patients behave in relation to more complex everyday tasks. By contrast, during medication OFF/not-ON states, the slow gait speed value as obtained in the lab can well inform about how PD patients perform their slow walking

activities in their usual environment. We could also show a high discriminant validity of these findings because the respective opposite correlations did not show significant results (Supplementary Table S2).

These results are of clinical relevance as they suggest that straight walking at preferred speed in the lab may be substituted by alternative measurements if the aim is to collect information relevant for everyday life of PD patients at ON state. However, even if the most suitable assessment is performed, only about 40% of home gait speed could be explained by the lab assessment. This means that lab tests generally explain less than half of the home performance and, consequently, monitoring of daily-life gait seems to be of utmost relevance for a comprehensive understanding of PD gait in usual environments [21].

We also compared gait speed from real-life settings with the UPDRS measurements, which are based on a one-time physical examination. Only specific items of the UPDRS-III showed significant correlations with PD gait performance in everyday life. Comparably similar results were observed in a previous study [48], where no significant correlation was found among the entire UPDRS subgroups, but gait features showed significant correlations with specific items of the UPDRS-III, mostly related to gait. Therefore, it could be an advantage to assess PD mobility using objective and targeted parameters from lab and home-based tools.

The present study has some limitations. First, the sample size was relatively small. Second, unsupervised assessments were performed for only one day. We based this choice on the following considerations: a previous study [49] evaluated the repeatability of sensor based assessments during two consecutive days and found that gait parameters were highly comparable between the two days. In addition, home and lab measures demonstrated strong discriminatory power in detecting impaired motor function in PD in another study, where unsupervised assessment was performed during one single day [22]. Still, further studies with several days of measurements are needed to capture a more granular picture of daily mobility [13]. Third, ON and not-ON states during daily-life activities were defined based on diary entries. Future studies should focus on a set-up that includes clearly defined OFF states (e.g., mornings, before medication intake).

We did not consider WBs shorter than 15 s, because we aimed to analyze steady-state gait and compare an equal number of steps with the lab capacity. However, in everyday situations, there are many shorter WBs occurring within the home or in-door conditions (<10 s) [11,37]. Since daily-living gait often takes place by using very short WBs, such bouts should also be considered in future analyses. Finally, the intra-subject variability of gait speed was considered only in the calculation of gait speed percentiles and not directly from the increment and decrement for each subject. Since it could be an interesting biomarker for the investigation of PD mobility, such analysis should be considered in further studies.

CONCLUSIONS

In-lab and daily-living testing with wearables can capture complementary aspects of PD, and substantially add to clinical evaluation and patient management. We highlighted which specific laboratory tests can better represent gait speed at home. This can be useful for clinicians, since it is possible to remotely assess the capacity of the patients in their domestic environment, and protect more vulnerable people, especially during the COVID-19 pandemic. On the other hand, if clinicians decide to perform only supervised tests, they know which functional tests are more indicative of patients' daily-life performance. Another relevant highlight is the importance of including two different methods (percentiles of the total gait distribution and WB types) in the analysis of home-collected gait speed, for a more detailed representation of PD daily-life performance. Improving the understanding of gait disabilities with additional information about daily-life performances from IMUs could enhance clinical care, design personalized interventions and overcome limitations concerning questionnaires and self-reported diaries.

Supplementary Material

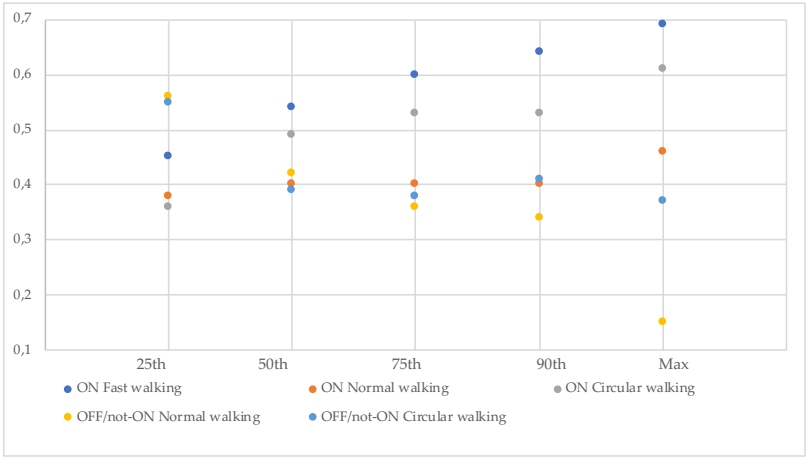

Figure S1. Correlation of ON and OFF/not-ON state between the lab and the most relevant percentiles of domestic environment.

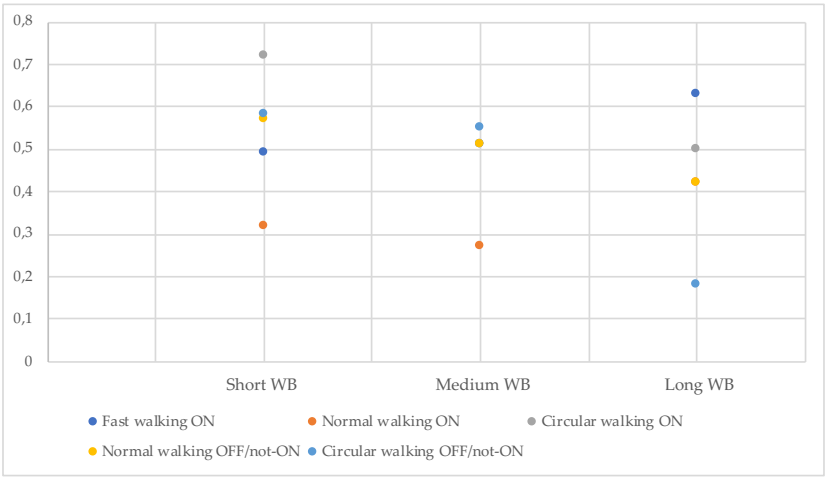

Figure S2. Correlation of ON and OFF/not-ON state between the lab and the most relevant WBs of domestic environment.

Table S1: Demographic data of the patients included and excluded in the analysis

Variables	**Included patients (27)**	**Excluded patients (12)**	***p* value**
Male: female	16:11	8:4	-
Age [years]	69 [64 : 73]	65 [61 : 74]	0.13
Disease duration [years]	6 [3 : 9]	4 [2 : 8]	0.3
Disease onset [years]	64 [57 : 69]	60 [53 : 69]	0.41
H&Y stage	**ON**: 2	2	0.39
	OFF: 2	2	0.06
UPDRS I	2 [1 : 4]	1 [1 : 2]	0.13
UPDRS II	7 [3 : 10]	4 [4 : 7]	0.23
UPDRS III	**ON**: 12 [8 : 20]	14 [8 :21]	0.5
	OFF: 22 [15 : 31]	22 [14 :33]	0.72
UPDRS IV	2 [0 : 3]	2 [0 : 3]	0.51
Total LED [mg]	580 [400 : 770]	540 [380 : 617]	0.36

Results are expressed in median and interquartile range [IQR].

Table S2: Correlation of ON and OFF / not-ON states between the lab and the domestic environment

LAB	Straight walking fast pace			Straight walking normal pace						Circular walking					
	ON			ON			OFF			ON			OFF		
HOME	P	r	R^2	P	r	R^2	P	r	R^2	P	r	R^2	P	r	R^2
ON	25^{th}	0.45 *	20	25^{th}	0.38	14	25^{th}	0.38 *	14	25^{th}	0.36	13	25^{th}	0.43 *	18
	Max	0.69 ***	30	Max	0.40 *	16	Max	0.5*	13	Max	0.61 **	39	Max	0.57 **	27
Not-ON	25th	0.49 *	24	25th	0.44 *	19	25^{th}	0.56 **	33	25th	0.55 **	30	25^{th}	0.55 **	31
	Max	0.47 *	22	Max	0.26	7	Max	0.15	2	Max	0.41 *	17	Max	0.37	14

Degrees of correlation (r) and coefficients of determination (R^2) between lab tests and most relevant percentiles (P) of gait speed in the domestic environment during ON and OFF / not-ON medication state. *p<0.05, **p<0.01, ***p<0.001.

Table S3: Characteristics of WB during ON and OFF / not-ON medication states.

	ON		**Not-ON**		***p value***
Time of ON and not-ON [hours]	5	[4 : 6]	2.5	[2 : 3]	<0.001
Walking time [%]	15.3	[10.4 : 26.3]	15.8	[9.3 : 18.7]	0.48
Total Short WB [N]	26	[11 : 42]	12	[7 : 23]	0.002
Short WB [N/h]	5.7	[3.4 : 7.5]	4.3	[2.8 : 9.4]	0.67
Total Medium WB [N]	9	[7 : 14]	5	[1 : 7]	<0.001
Medium WB [N/h]	2.3	[1.4 : 2,9]	1.7	[0.5 : 2.6]	0.27
Total Long WB [N]	5	[2 : 9]	2	[1 : 4]	0.024
Long WB [N/h]	1.3	[0.7 : 1.7]	0.7	[0.3 : 1.6]	0.51

Values are expressed in median [IQR].

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CHAPTERS 4.

WEARABLE HEALTH TECHNOLOGY TO QUANTIFY THE FUNCTIONAL IMPACT OF PERIPHERAL NEUROPATHY ON MOBILITY IN PARKINSON'S DISEASE: A SYSTEMATIC REVIEW

This Chapter was adapted from the published work:

Corrà MF, Warmerdam E, Vila-Chã N, Maetzler W, Maia LF. Wearable Health Technology to Quantify the Functional Impact of Peripheral Neuropathy on Mobility in Parkinson's Disease: A Systematic Review. Sensors (Basel). 2020 Nov 19;20(22):6627. doi: 10.3390/s20226627.
Full Open Access publication

ABSTRACT: The occurrence of peripheral neuropathy (PNP) is often observed in Parkinson's disease (PD) patients with a prevalence up to 55%, leading to more prominent functional deficits. Motor assessment with mobile health technologies allows high sensitivity and accuracy and is widely adopted in PD, but scarcely used for PNP assessments. This review provides a comprehensive overview of the methodologies and the most relevant features to investigate PNP and PD motor deficits with wearables. Because of the lack of studies investigating motor impairments in this specific subset of PNP-PD patients, Pubmed, Scopus, and Web of Science electronic databases were used to summarize the state of the art on PNP motor assessment with wearable technology and compare it with the existing evidence on PD. A total of 24 papers on PNP and 13 on PD were selected for data extraction: The main characteristics were described, highlighting major findings, clinical applications, and the most relevant features. The information from both groups (PNP and PD) was merged for defining future directions for the assessment of PNP-PD patients with wearable technology. We established suggestions on the assessment protocol aiming at accurate patient monitoring, targeting personalized treatments and strategies to prevent falls and to investigate PD and PNP motor characteristics.

Keywords: peripheral neuropathy; Parkinson's disease; wearable health technology; functional assessment.

INTRODUCTION

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder, clinically defined by the presence of resting tremor, rigidity, and bradykinesia [1]. These

features are collectively referred to as motor symptoms and mostly related to loss of dopaminergic neurons in the pars compacta of midbrain substantia nigra. Alpha-synuclein positive intra-cytoplasmatic inclusions, known as Lewy bodies, are the pathological hallmark of the disease [2]. As the disease progresses, motor disturbances represent considerable illness burdens. Deficits in balance and gait are common and disabling features that significantly increase the patient's risk of falling [3] and the managing of daily living activities [4].

PD is also characterized by strong clinical and neuropathological evidence of systemic involvement. The presence of Lewy bodies in several other nervous structures, such as the nervous fibers in the skin, indicate that peripheral nervous system (PNS) involvement may be an intrinsic part in the PD pathological process [5,6]. Since the PNS is a target of alpha-synuclein deposition, it is plausible that intrinsic pathogenic features of PD may predispose to peripheral neuropathy (PNP).

PNP refers to any disorder of the PNS including single and multiple mononeuropathies, symmetrical involvement of nerves (polyneuropathies), or isolated involvement of sensory ganglia (ganglionopathies) [7]. It usually starts gradually and presents in the most common types a distal-proximal gradient, affecting first the feet and later the hands [8].

The occurrence of PNP in PD (PNP-PD) has been shown to be present in up to 55%, compared to 8% in the general population with comparable age [9–11]. Typical features of PNP include postural instability, muscle cramps, and numbness, of which the latter two are more prominent at distal part of the legs. As both PD and PNP pathologies are associated with these symptoms, the concurrence of peripheral involvement could be considered as an additional cause of motor deficits and general worsening in PD [12].

PNP can worsen the global functional mobility of patients, since neuromuscular factors (hip strength, ankle proprioception, and decreased peripheral sensation) have been linked to gait and balance difficulties [13]. It is, therefore, plausible to hypothesize that PD patients with PNP (PNP-PD) may develop more prominent gait and balance deficits and, consequently, be at risk of falling, injuries, and reduced quality of life [14].

Wearables are constituted of all mobile devices worn on the body (also called on-body sensors), such as inertial measurement units (IMUs), smartwatches, or Holter electrocardiogram monitors [15]. They provide objective and quantitative measures from controlled and unsupervised environments, allowing the development of accurate treatment plans and disease monitoring. In particular, data obtained from IMUs can successfully estimate spatial-temporal parameters and provide sensitive and objective information about motor deficits of various neurological pathologies, which nontechnological motor assessments often cannot identify. Mobility assessment with wearable health technologies are widely investigated in a variety of illnesses, particularly in PD, and allows high

sensitivity, accuracy, and reproducibility [16]. However, these methodologies are scarcely studied and have yet to be explored in PNP [17], although a small number of previous works using wearable sensors have successfully demonstrated motor and physical activity characteristics in PNP compared to controls [18,19]. Since the presence of PNP has only recently been considered related to PD, we were interested in understanding whether PNP-PD patients showed specific motor deficits, which can be measured with the use of wearable health technology. For such purpose, a preliminary review of literature performed by the authors showed no studies evaluating the functional impact of PNP in PD on mobility using wearables. Identifying specific gait and balance patterns in this specific subset of PNP-PD patients could provide additional information about gait and balance problems, which can be used to monitor and stratify patients, optimize treatment, prevent falls, and increase quality of life.

For this purpose, in this systematic review we investigated the methodologies (type, number, and location of wearables) mostly used and which parameters (or change of parameter) are the most relevant and clinically useful to characterize PD- and PNP-associated gait and balance deficits. Because of the lack of studies investigating gait and balance impairments in PNP-PD patients with wearables, we divided the search into two parts: We performed a systematic review on the assessment of PNP with wearable health technology and, separately, we reviewed the literature to characterize the use of wearables for PD. The authors defined the major results and conclusions from both searches (PNP and PD) based on the occurrence, significance, and clinical relevance in the included studies. Future directions for the assessment of PD patients with and without PNP phenotype with wearable health technology were then proposed. This study will help to accurately stratify and monitor PD- and PNP-associated functional deficits of gait and balance and target strategies to prevent falls. This could have an impact on the diagnosis and on the clinical approach of PD patients.

MATERIALS AND METHODS

Search Strategy

In this systematic review we adopted PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement methodology [20].

Pubmed, Scopus, and Web of Science electronic databases were searched in April 2020 to identify relevant papers based on their title and abstract. A combination of MeSH (Medical Subject Headings) terms and keywords were used in the search. Since the presence of PNP has only recently been considered related to PD, we were interested in understanding whether PNP-PD patients showed specific motor deficits, which can be measured with the

use of wearable health technology. However, a preliminary review of literature performed by the authors showed no studies evaluating the functional impact of PNP in PD on mobility. Therefore, because of the lack of papers on PNP-PD with wearables, two separate search strategies were used to find relevant papers:

(1) To investigate the main characteristics and the most relevant gait and balance features for studying PNP with wearable technology, the following keywords were used: “peripheral neuropathy” OR “polyneuropathy” OR “small fiber neuropathy” AND “wearable sensor” OR “wearable” OR “mobile health technology” OR “technology assessment” OR “body-worn sensors” OR “inertial sensor” OR “inertial measurement unit” OR “acceleromet*” OR “gyroscope” AND “mobility” OR “gait” OR “balance” OR “postural balance” OR “postural stability” OR “postural strategies”.
In addition, due to the lack of data on PNP with wearables, we performed a literature research to report, narratively and not systematically, other systems, tools, and relevant features coming from other movement analysis methods, used for the assessment of gait and balance in PNP.

(2) To investigate the main characteristics of wearable sensor assessments, and the most relevant gait and balance features in PD, the following keywords were used: “Parkinson” AND “wearable sensor” OR “wearable” OR “mobile health technology” OR “technology assessment” OR “inertial sensor” OR “inertial measurement unit” OR “acceleromet*” OR “gyroscope” AND “mobility” OR “gait” OR “balance” OR “postural balance”.
Unlike in PNP, wearable technology in PD is highly investigated. For this reason, we decided to select the already existing reviews from this search, to provide an overview of PD assessments with wearable technology. The completed search queries are provided in the Appendix A.

Selection Criteria

Research methodology for study selection, according to the PRISMA statement, are shown in Figures 1 and 2. Studies were excluded if they were: (1) not published in English, (2) published before January 2010, (3) not done in humans, (4) nonoriginal full-text manuscripts, (5) a case study or did not enroll >10 subjects, and (6) were out of topic with respect to the aims of the present study (i.e., not regarding PNP, focusing on the validation of algorithms or on machine learning classification, not investigating gait and balance characteristics and parameters, or studying other types of wearables).
Only original papers were considered for the first literature search. For the second part about PD, reviews that were found with the above search criteria were screened.

After the definition of the selection criteria by all the authors, the selection process was performed by one author. Doubts were decided consensually by three authors.

Works prior to 2010 were not included because wearables were scarcely used for assessing PNP mobility before this date and, secondly, we aimed to focus on the most accurate technology and software, which was mostly developed in this last decade.

In this work, wearables include all the on-body fixed sensors (tightly fixed to the body with straps, Velcro, or tape) that incorporate at least an accelerometer, gyroscope, or magnetometer or a combination of those and that can extract mobility-related parameters that have been mostly used in research and clinical trials. Ambient sensors were not included in the search because they are not yet commonly used to measure mobility. Therefore, there was not enough literature available to provide any well-founded conclusion about the use of these sensors.

Data Extraction

Upon manuscript selection, the following information was extracted and collected: the type and number of participants and socio-demographic characteristics, the type and location of the wearable sensor(s) used, the main extracted features and the major findings of the study.

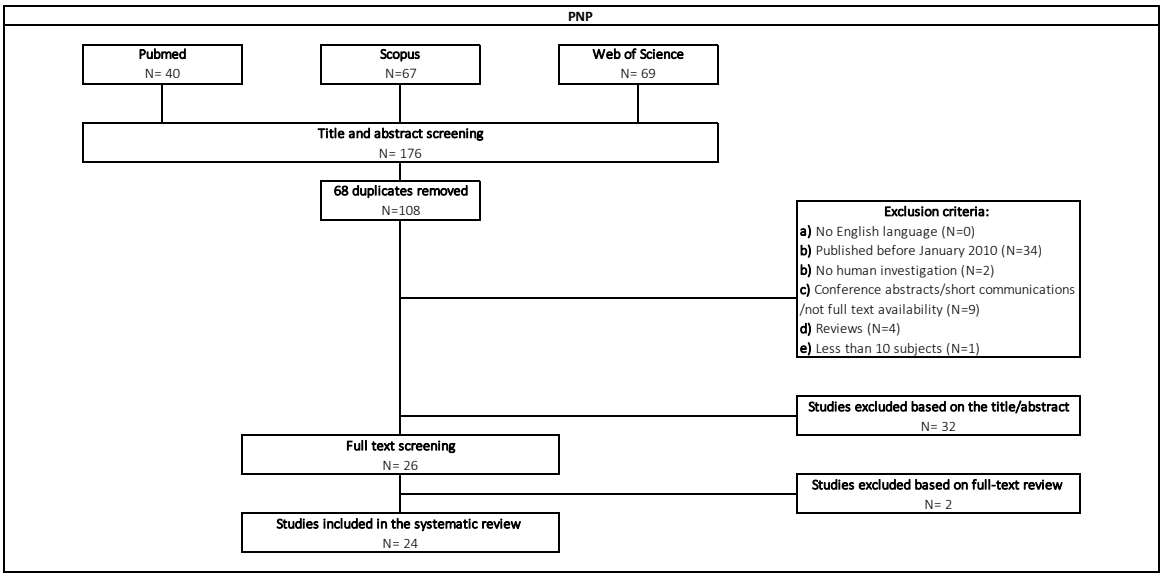

Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart for peripheral neuropathy (PNP) and wearable technology assessment.

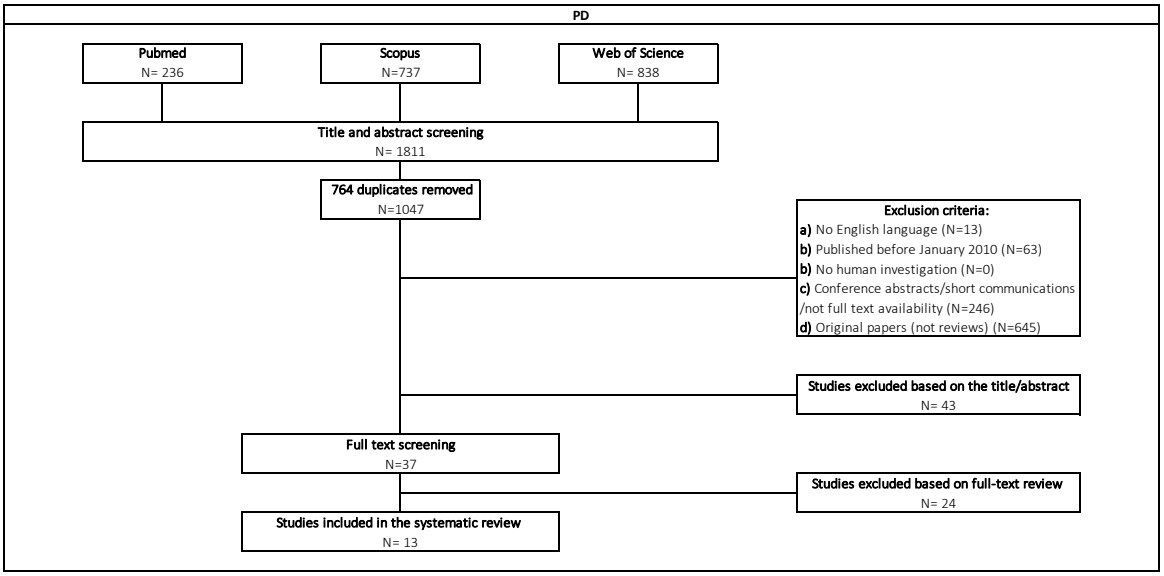

Figure 2. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart for Parkinson's disease (PD) and wearable technology assessment.

RESULTS

For the PNP search part, an initial database search identified 176 studies that were potentially eligible for inclusion in this review. After duplicates were removed, 108 abstracts were screened. From these, 26 full texts were selected, of which 24 studies were included in this review (Figure 1). For the PD search part, a total of 1811 studies were extracted by the search detailed above. The screening of titles and abstracts removed 1774 studies due to previously stated exclusion criteria. The remaining 37 selected reviews were screened in their full-text versions to assess their inclusion in the review. Finally, 13 reviews were included in this study (Figure 2). A summary of the main characteristics of the included PNP papers and PD reviews are reported in Tables 1 and 2.

Sample Population Characteristics

Sample population characteristics and sizes varied across the included studies on PNP. The subjects enrolled in these studies consisted of healthy adults (with mean age between 24 and 78 years) and PNP patients with the following etiology: diabetic peripheral neuropathy (DPN) (70.8%), chemotherapy-induced peripheral neuropathy (CIPN) (12.5%), combined DPN and CIPN (8.3%), chronic inflammatory demyelinating polyneuropathy (CIDP) (4.2%), and lower-limb PNP without specific etiology (PNP-LL) (4.2%). Sample sizes ranged from 19 to 434 subjects. With regard to PD sample characteristics, the selected reviews described a wide range of participants: for free-living recording at home or home-

like environment, sample size ranged between 1 to 467 participant(s) and the majority (49%) of studies were between 10 and 49 participants [21–23]. For the lab assessments, the majority of the studies ranged from 5 to 67 participants and four reviews reported studies over 100 study participants.

Sensor Type and Placement

Peripheral Neuropathy

Multiple wearable sensor types were used within the included articles to assess measures of gait and postural stability in PNP patients. Among the 24 included articles, the most commonly used inertial sensors included a tri-axial accelerometer and a tri-axial gyroscope (83.3% of the studies): LegSys. and BalanSens. (BioSensics), used, respectively, for gait and balance assessment; the Opal v1 (APDM) and the Physilog® (BioAGM) for balance assessment; the GaitMeter. for gait assessment; and the mHT (mHealth Tecnologies) for both gait and balance assessment. Accelerometers only were used in two studies: PAMSys.(BioSensics) and DynaPort Mini-Mod (McRoberts BV). One study used a gyroscope-based sensor (SwayStar device, Balance International Innovations GmbH) for balance assessment [24]. Sampling frequencies between 50 and 200 Hz were used to acquire the signals. The most commonly used sampling frequency was 100 Hz. Several sensor placements and numbers of wearable sensors were used, depending on the task and on the type of assessment. Among the 16 included studies analyzing gait in PNP, four papers (25%) used one sensor, four studies (25%) analyzed gait with sensors on both shanks (two sensors), one paper (6.25%) used four sensors, and six studies (37.5%) assessed gait with five wearable sensors placed on thighs, shanks, and lower back. One study did not report sensor placement (6.25%). Postural stability was assessed in 13 studies: Three studies (23%) used one sensor on the lower back, five studies (38.6%) used two sensors, and two studies (15.4%) used three sensors on both shanks and lower back. The remaining three studies (23%) utilized five sensors (Figure 3, Table 1). Postural stability was assessed in 13 studies: Three studies (23%) used one sensor on the lower back, five studies (38.6%) used two sensors, and two studies (15.4%) used three sensors on both shanks and lower back. The remaining three studies (23%) utilized five sensors (Figure 3, Table 1).

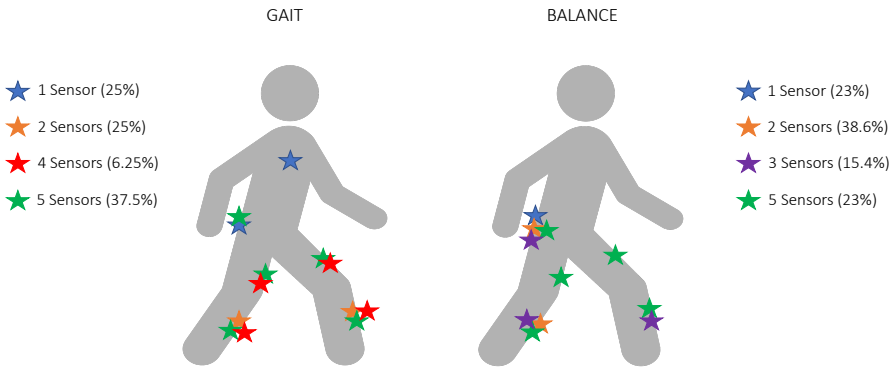

Figure 3. Anatomical representation of sensor placement for gait and balance assessment in patients with polyneuropathy (PNP).

Parkinson's disease

There is currently no consensus available on the optimum number and placement of sensors to measure PD symptoms. All reviews included that evaluated sensor number and placement showed that the majority of the studies used one sensor placed on the lower back (at lumbar vertebrae level L3, L4–L5, sacrum, or waist) or on the dominant lower limb (thigh, shank, ankle, or foot). Single sensors seemed suffciently robust for all applications: For gait assessment at home, one sensor was used in 28% to 47% of the studies [21–23], while for gait evaluation in the laboratory it ranged from 44% to 69% [25,26]. Not surprisingly, for balance assessment the use of one sensor, and specifically on the lower back, was preferred in 77% to 100% of the studies included in the reviews [26–28]. Other most commonly used sensor placements for PD were on both wrists or lower limbs (in 30% of studies) or on lower back and both lower limbs (in 14% of studies) for the home assessment and at both lower limbs (8% of the studies) for laboratory assessment (Table 2).

Parameters and Main Outcomes

Peripheral Neuropathy

We included 24 original full-text manuscripts: Eleven studies (45.8%) investigated gait, eight (33.4%) analyzed balance, and five (20.8%) evaluated both gait and balance in PNP patients. Gait was assessed mainly during a straight walking task at preferred gait speed, with a distance varying from 7 to 50 m. In two studies patients were asked to perform a 90 turn during walking [29,30]. Several parameters were calculated from the signals acquired

through the wearable sensors. The most commonly reported parameters computed from the filtered signals were spatiotemporal gait parameters: gait speed (m/s), stride and step length (m), stride and step time (sec), number of steps, double limb support time (%), and cadence (steps/min). Coefficient of variation (CV) of gait speed and stride length and time (%) was calculated in eight studies [29–36]. Gait speed initiation, number of steps, and total distance required to reach steady-state walking were studied in four papers [34,35,37,38]. Duration (%) and number of walking bouts were extracted in one study [18].

Clinical trials among the included papers did not show any statistically significant changes in the gait parameters when comparing pre- and post-intervention. Najafi [39] analyzed gait differences between intervention and control groups after plantar electrical stimulation in DPN patients and Schwenk et al. [33] evaluated gait after a new interactive training in CIPN subjects. Nevertheless, the effect size of these studies suggested the presence of a moderate to large improvement of cadence and gait speed post-treatment. In contrast, Caronni [40] compared the responsiveness to rehabilitation in a group of PNP patients and found a statistically significant difference in gait speed between groups ($p = 0.001$, Table 1). Spatiotemporal parameters were significantly different between PNP patients and healthy controls only in studies investigating gait under more challenging conditions. Kang et al. [32] described a statistically significant difference between DPN and healthy participants in the coefficient of variation of gait speed and stride length during dual-task gait. De Bruin et al. [41] found significant differences in speed, step length, and cadence when comparing DPN patients during dual task walking on paved trajectories compared to single-task. Another study by Kang [42] showed improvement in stride velocity, stride length, and double limb support (%) during dual-task and fast walking, compared to single-task, after plantar mechanical stimulation. Differences from controls were found in step time, cadence, and gait speed but not in stride length in a study by Esser et al. [17], and gait speed was also 10% decreased in DPN group compared to controls in a study by Ling et al. [31]. Another important result was pointed out by Najafi et al. [34], who found differences in spatiotemporal parameters only during long distances, especially in gait variability and in double support time, when comparing DPN patients with controls. These differences were more pronounced during barefoot walking.

Balance and postural stability were investigated through numerous tasks. The most frequently used task in all 13 studies was the double leg stance performed in different conditions:

(1) Position of feet: Standing balance was assessed with feet together in eight (61.5%) studies, feet apart (spaced shoulder width) in two studies (15.3%), and both feet positions in one paper (7.6%), while two papers (15.3%) did not specify the position of the feet. In

two studies patients were also asked to perform a semi-tandem position [33,43], while one other study introduced a detailed balance test protocol with single leg stance [24].
(2) Open and closed eyes: Twelve studies (92.3%) analyzed balance with both open and closed eyes, and one study only used eyes-open condition [44].
(3) Foam: Two studies used a foam surface (height 10 cm, density 25 kg/m3) to analyze balance [24,43]. The other papers only performed balance tasks on firm surfaces.
Other tools to assess postural stability were clinical tests such as the functional reach test [45]. Functional tests (to investigate functional mobility, addressing both gait and balance characteristics) were performed in three selected studies [40,42,45]. They applied the timed up-and-go (TUG) test. This test was split by Caronni et al. [40] into five subphases, and the duration of each phase was measured, as well as the total TUG test duration.
The included studies reported multiple outcomes of standing balance and postural stability that were calculated from the signals provided by the wearable sensors (Table 1). Of these outcomes, the most commonly reported measures included center of mass (COM) sway (cm2), defined as total sway (in seven studies, 53.8%), and related parameters (anterior-posterior (AP) and medio lateral (ML) sway (cm)). These parameters were also reported in three studies analyzing gait to investigate balance control during walking and gait initiation [34,35,38]. In addition, ankle sway (deg2), hip sway (deg2), and COM sway area (m2) were calculated in six papers (46.1%). Center of gravity (COG) sway (cm2), COG AP, and COG ML (expressed in cm) were calculated in one paper [46]. Other parameters were root mean square (RMS, m/s2), trunk acceleration, and trunk jerk (m2/s3) [40,47]; postural coordination of upper and lower body (defined as the reciprocal coordination between hip and ankle motions) [36]; roll and pitch velocity (deg/sec) and roll and pitch angle (deg) [24]. Further parameters were local (in short time intervals, sec) and central (in long time intervals) control balance strategies [46], and cross-correlation function (CCF) of angular velocity to investigate the coordination of human movements [47]. A significant reduction in COM sway area (a parameter of postural sway) was shown by Schwenk et al. [33] and Grewal et al. [48] after an interactive sensor-based balance training and by Yalla et al. [45] after an intervention on postural stability with an ankle foot orthosis. These results were found during balance tasks with open eyes, while, interestingly, no significant reduction was found during closed-eyes condition. In contrast, changes of the parameters COM sway area and ML sway area were significant after a virtual reality intervention with eyes-closed and -open conditions [36].

Parkinson's disease

In PD, a multiplicity of parameters derived from inertial sensors could be described. For the purpose of this review, parameters from the upper part of the body (upper limb) were not

considered. The included reviews listed a series of most relevant spatiotemporal parameters representative of five domains (pace, variability, rhythm, asymmetry, and postural control), which included stride length, stride velocity, cadence, double support time [49,50], and turning velocity [51] followed by step time variability [26,49] and step height, reaction time, and gait cycle duration [52]. Frequency based measures were dynamics in trunk movement during gait, turning and smoothness [53], harmonic ratio, amplitude, slope and width of dominant frequency, peak trunk horizontal velocity, and phase coordination index of gait cycle [26]. Number of steps, single versus multiple step response, turning duration, turn-to-sit duration, and sit-to-stand and stand-to-sit time- and amplitude-based measures were reported to be important features to determine gait impairment [52]. In more detail, PD patients have been shown to have slower gait, less foot clearance, smaller step lengths, lower turning velocity, lower cadence, and lower peak trunk rotation compared to controls [49,51]. Turning velocity, cadence, and peak trunk rotation were associated with disease progression [54]. Another important parameter in PD is gait variability, also referred to as unsteadiness and arrhythmicity of stepping [55]. Increased gait variability can be seen throughout the disease, and the magnitude of the variability tends to increase with disease severity [49].

Home assessment may have greater ecological validity and gives a true picture of the burden of disease [15]. Parameters that may be particularly relevant for this assessment type are walking bouts (total number of walking bouts, median number of steps per bout, bout duration), turns per hour during the day, duration of each turn, number of steps per turn, peak and average rotational turning rate, and variability of these measures throughout the day and week [22,23].

Regarding standing balance and postural stability, often used parameters were postural sway velocity, RMS accelerations, and jerk [28]. Parameters that may discriminate most effectively between PD and controls are sway area, sway velocity, jerk index, sway amplitude and range of acceleration signals (time domain), and frequency dispersion and centroidal frequency [27,49] (Table 2).

All these features are able to differentiate between PD and healthy controls (HC) at early stage [26,49], different PD stages [28], different medication states in advanced PD, and PD progression (in particular sway dispersion and sway velocity) [49]. Postural sway is also a good measure of balance control to be used as a primary outcome for interventions [49].

TABLE 1 and 2. Summary of the major characteristics of the PNP and PD studies that met the inclusion criteria.

TABLE 1. Summary of the major characteristics of the PNP studies that met the inclusion criteria.

REF	POPULATION (Mean age ± SD)	SENSORS (number and type)	SENSOR PLACEMENT	ASSESSMENT PROTOCOL	PARAMETERS EXTRACTED/INVESTIGATED/OUTCOMES	MAIN FINDINGS
Ling et al, 2020 [26]	• 12 DPN + DFU (55.6 ± 3) • 27 DPN (64.3 ± 1) • 47 Healthy controls (62.9 ± 2)	5 Inertial sensors (ACC, GYR and MAG) (LegSys™, BioSensics) Freq: 100 Hz	• Thighs • Shanks • Lower back	Straight walking test at preferred speed for 10 meters on a flat floor	• Gait speed and gait speed unsteadiness, stride length and stride length unsteadiness, gait cycle time, double support and double support limp, step length limp, gait symmetry	People with DPN and DFUs wearing offloading devices have poorer gait function compared to controls. DFUs and offloading devices further deteriorate gait beyond DPN, specifically for performance in gait speed, stride length and gait cycle time. Compared to controls, DPN showed 10% decreased in gait speed and increased stride length of 48%.
Kang et al, 2020 [32]	• 38 DPN (72.6 ±5) • 33 Healthy controls (77.9 ± 8)	5 Inertial sensors (ACC, GYR and MAG) (LegSys™, BioSensics) Freq: 100 Hz	• Thighs • Shanks • Lower back	Straight walking test at preferred speed for 12 meters on a flat floor at two conditions: during single and dual (cognitive) task	• Number of steps and distance to reach steady-state gait • Gait speed and body sway in the mediolateral direction in the gait initiation phase and steady-state gait speed.	For both single-task and dual-task gait conditions, number of steps, distance, and mediolateral body sway were still significantly greater for the DPN group than for the CON group. Gait initiation steps and dynamic balance may be more sensitive than gait speed for detecting gait deterioration due to DPN.
Kang et al, 2020 [27]	44 DPN + CIPN: • 25 PNP with no cognitive impairment (66.5 ± 9) • 19 PNP with cognitive impairment (68.5 ±9)	2 Inertial sensors (ACC, GYR and MAG) (LegSys™, BioSensics) Freq: 100 Hz	• Shanks	Straight walking test at preferred speed for 12 meters on a flat floor at two conditions: during single and dual (cognitive) task	• Coefficient of variation (CV) of gait speed, stride length and stride time • Spatio-temporal gait parameters: gait speed, stride length and stride time	During dual-task walking, between-group differences were significant for gait variability for gait speed and stride length (51.4% and 71.1%, respectively; p = 0.014 and 0.011, respectively). The presence of cognitive impairment exacerbates the risk of falls in people with PN.
Kang and Najafi, 2020 [34]	• 49 PNP (DPN+ CIPN) (68.5 ± 7)	1 accelerometer (ACC) (PAMSys™, BioSensics LLC) Freq: 50 Hz	• Chest	48-hour period recording	• Durations of standing posture • Sedentary posture • Total number of walking bouts • Number of total steps	People with PN and low concern about falling tended to have more activity, but people with PN and high concern about falling tended to have less activity. Furthermore, the duration and amount of being active (i.e., walking bout and total step counts) may predict the level of concern about falling, and thus may be used as eHealth targets and strategies for fall risk assessment among people with PN.

Zahiri et al, 2019 [68]	• 84 subjects with cancer (CIPN+ and CIPN-) (71.1 ± 9) • 57 Healthy controls (69.5 ± 9)	5 Inertial sensors (ACC, GYR and MAG) (LEGSys™and BalanSens™; Biosensics LLC) Freq: not reported	•Shanks •Thighs • Lower back	• Gait assessment: single-task (no cognitive distraction) over 15 meters at a self-selected speed. • Balance: double leg stance 30 s with feet close together during eyes-open and eyes-closed situations.	• Gait parameters: stride velocity, stride length, stride time and double support time. • Balance parameters: area of ankle sway, area of hip sway, area of center of mass (CoM) sway, and CoM sway in the medial-lateral (ML) direction.	The deterioration in gait parameters was more pronounced in the CIPN+ than the CIPN- subgroup, when compared to the control group. CIPN+ on average had 8% and 18% slower stride velocity compared to the CIPN- and control groups, respectively. Stride velocity was also on average 11% slower in CIPN, when compared to control. Similar trends were observed for other gait parameters of interest. Results also suggest high visual dependency in the CIPN+ subgroup. The negative impact of CIPN on motor-performance is confirmed with the largest effects on ankle stability and stride time. Vibration perception threshold (VPT) is a predictor of motor deterioration and may be used to determine the severity of CIPN symptom
Kang et al, 2019 [39]	• 30 DPN (68.1 ± 9)	• Gait assessment: 5 Inertial sensors (ACC, GYR and MAG) (LEGSys™, Biosensics) • Balance assessment: 2 Inertial sensors (ACC and GYR) (BalanSens™, Biosensics) Freq: not reported	Gait assessment: • Shanks • Thighs • Lower back Balance assessment: • Dominant leg • Lower back	• Gait: 10 m walking test at normal and fast pace, and at two conditions: single and dual tasks. • Static balance: (i) double leg stance for 30 s with feet together with eyes open and eyes closed (EC). (ii) semi tandem stance for 30 s • Global functional mobility: TUG test	• Gait parameters: stride velocity, stride length, stride time and double support time. • Balance parameters: area of ankle sway, area of hip sway, area of center of mass (CoM) sway, and CoM sway in the medial-lateral (ML) direction.	Daily use of plantar mechanical stimulation through a micro-mobile foot compression device installed in a shoe insole is effective for improving vibration perception, which likely results in improvements in some balance outcomes and gait parameters. Key findings were improvements in plantar sensation in the foot, CoM sway in the ML direction during quiet standing and stride velocity and other spatiotemporal gait parameters in dual task condition after using the wearable foot compression device for four weeks.
Fino et al, 2019 [36]	• 216 CIPN+ (63.0 ± 6) • 218 CIPN- (62.2 ± 6) • 49 Healthy controls (63.3 ± 6)	1 Inertial sensor (ACC and GYR) (Opal v1, APDM) Freq: 128 Hz.	• Lower back	Double leg stance test with eyes open for 30 s, feet apart	AP-sway, ML-sway, or resultant sway	Cancer survivors had worse sway than healthy control subjects in components related to sway magnitude and mediolateral frequency of sway, but no difference in the component related to resultant / AP sway jerk and frequency. Cancer survivors who reported neuropathy were more likely to have higher resultant / AP sway frequencies and jerk than asymptomatic survivors, while survivors who reported a fall were more likely to have lower frequencies of mediolateral sway than non-fallers. Neuropathy influenced the associations between specific characteristics of sway and falls, which may have implications for fall prevention interventions.

Caronni et al, 2019 [38]	• 25 PNP-LL (76.5 ± 6)	1 Inertial sensor (ACC and GYR) (mHT, mHealth Technologies) Freq: 100 Hz	• Lower back	• Gait: 10 m walking test and TUG test repeated five times each. • Static balance: double leg stance for 30 s with (i) feet apart (FA) and eyes open (EO), (ii) feet apart and eyes closed (EC), (iii) feet together (FT) and eyes open an (iv) feet together and eyes closed.	• Gait: 5 subsequent phases of TUG test: sit to stand (STS), walk 1 (W1), turn 1 (T1), walk 2 (W2) and turn and sit (TAS); duration of each phase and total TUG duration (TTD); mean vertical angular velocity during turn 1 and during TAS • Root mean square (RMS), trunk acceleration (Trunk acc) and trunk jerk (Trunk jerk).	After rehabilitation, patients with PN-LL consistently improved straight walking, walking along curved trajectories and transfers, with no apparent modification of static balance. Four gait measures (i.e. gait speed, angular velocities during TUG) and the TTD showed a large improvement after rehabilitation. The improvement was medium for the walking phases of the TUG test (i.e. W1, T1 and W2) and TUG transfers (i.e. STS and TAS).
Findling et al, 2018 [19]	• 11 CIDN (chronic inflammatory demyelinating polyneuropathy) (61.1±11) • 10 not inflammatory PNP (68.5 ± 11)	1 gyroscope SwayStar device (GYR) (Balance International Innovations GmbH) Freq: 100 Hz	• Lower back	12 stance tasks: • **4** double leg tests with the feet spaced shoulder width apart; • 4 tasks with eyes open±on a normal surface and on a foam surface (height 10 cm, density 25 kg/m3)±and eyes closed. • **3** single leg stance tasks with eyes open, 2 on a normal surface (right and left leg) and 1 on the foam surface. • **1** task with single leg standing. 5 tasks for dynamic balance: • 8 steps tandem gait • 3m walking on heels • 3m walking pitching the head up and down • 3m walking with eyes closed and 8m walking with eyes open	Global balance control index (BCI); trunk sway and trunk velocity	CIDP patients have reduced ability to decrease trunk sway with lower gait speed. A similar effect was noted for pitch velocity walking eyes closed. This is possibly associated with an increased risk of falls
Esser et al, 2018 [48]	• 17 DPN (63 ± 9) • 42 Healthy controls (61 ± 4)	1 inertial sensor (ACC and GYR). Freq: 100 HZ	• Lower back	• Gait: 10m at normal and fast pace	Step time, cadence, stride length, walking speed	A single IMU used in clinical setting has the potential to discriminate patients with DPN compared to healthy controls. Walking speed was the most sensitive parameter, while no significant differences were found in stride length compared to controls.

Najafi et al, 2017 [53]	• 28 DPN: 17 intervention group (56 ± 5) • 11 Healthy controls (64 ± 10)	• Gait assessment: 2 Inertial sensors (ACC, GYR and MAG)(LEGSys™, Biosensics) • Balance assessment: 2 Inertial sensors (ACC and GYR) (BalanSens™, Biosensics) Freq: not reported	Gait assessment: • Shanks Balance assessment: • Dominant leg • Lower back	• Gait: 10m at normal and fast pace • Balance: double stance for 30 s with feet close together (without touching), with eyes open (EO), and eyes closed (EC).	• Gait: Stride velocity, stride time, stride length and cadence. • Balance: COM anterior-posterior (AP) sway, medial-lateral (ML) sway, and total sway area	No differences were observed between the groups for baseline characteristics or for motor performance including postural sway and spatiotemporal parameters of gait. However, the majorities of measurable metrics were improved post-treatment in the intervention group with no significant changes in the control group. This study suggests that daily home use of plantar electrical-stimulation may be a practical means to enhance motor-performance and plantar-sensation in people with DPN.
Schwenk et al, 2016 [28]	• 22 CIPN (70.3 ± 8)	• Gait assessment: 4 Inertial sensors (ACC, GYR and MAG) (LEGSys™, Biosensics) • Balance assessment: 3 Inertial sensors (ACC and GYR) (BalanSens™, Biosensics) Freq: not reported	Gait assessment: • Shanks •Thighs Balance assessment: • Shanks • Lower back	• Gait: 10m at normal pace • Balance: double stance 30 s with feet close together (without touching), with eyes open (EO), and eyes closed (EC), and semi-tandem position with EO.	• Gait: gait speed and variability • Balance: COM AP sway and ML sway; hip sway and ankle sway	ML CoM sway, hip sway, and ankle sway were reduced in the intervention group compared to control group during balance assessment with feet close together and EO. Significant reductions in postural sway parameters were also found during the more challenging semi-tandem position, except for ankle sway. Older cancer patients with CIPN can significantly improve their postural balance with specifically tailored, sensor-based exercise training.
Toosizadeh et al, 2015 [40]	• 18 DPN (65 ± 8) • 18 Healthy controls (69 ± 3)	2 Inertial sensors (ACC and GYR) (BalanSens™, Biosensics) Freq: not reported	• Ankle • Hip	2 Romberg balance trials (with open and closed eyes) for 15 s	Center of gravity (COG) sway (total sway) and COG (AP) sway, COG (ML) sway; local- (in short time-intervals) and central- (in long time intervals) control balance strategies.	The rate of sway within local-control was significantly higher in the DPN group by 49%, which suggests a compromised local-control balance behavior in DPN patients. Unlike local-control, the rate of sway within central-control was 60% smaller in the DPN group, which suggests an adaptation mechanism to reduce the overall body sway in DPN patients. In the lack of sensory feedback cueing, DPN participants were highly unstable compared to controls. However, as soon as they perceived the magnitude of sway using sensory feedback, they chose a high rigid postural control strategy, probably due to high concerns for fall, which may increase the energy cost during extended period of standing.

Grewal et al, 2015 [62]	• 35 DPN: 19 intervention group (62.5 ± 7) • 16 Healthy controls (64.9 ± 8)	5 Inertial sensors (ACC, GYR and MAG) (LEGSys™, Biosensics LLC) Freq: 100 HZ	•Shanks •Thighs • Lower back	Double leg stance for 30 s with open and closed eyes and feet together	COM sway, COM AP, COM ML sway, Hip sway.	On average, the CoM sway area for the intervention group (IG) was reduced significantly by 58.31% compared to a reduction of 7.8% in the control group (CG). The IG showed a significant reduction in the ML CoM sway; similarly, significant reductions were observed for the hip and ankle sway in the IG compared to the CG. During balance assessment with closed eyes, the IG achieved a reduction in CoM sway of 62.68%; however, none of the sway components (AP, ML or CoM area) reached significance. People with DPN can significantly improve their postural balance with diabetes specific, tailored, sensor-based exercise training
Yalla et al, 2014 [37]	• 30 DPN (73 ± 6)	5 Inertial sensors (ACC and GYR) (BalanSens™, BioSensics LLC) Freq:100 Hz	•Shanks •Thighs • Lower back	• 6 double stance of 30 s trials (2 for each footwear condition during eyes-open and eyes-closed) with their arms crossed, feet positioned close to each other without being in contact. • Dynamic balance: Functional reach task • Global functional mobility: TUG test	Ankle, hip, and COM sway	The orthoses reduced center of mass sway on average by 49.0% and 40.7% during eyes-open balance trials. The reduction was amplified during the eyes-closed trials with average reductions of 65.9% and 47.8%, compared to barefoot and 'shoes alone' conditions. Ankle foot orthoses reduced postural sway and improved lower extremity coordination in the elderly participants without limiting their ability to perform a standard activity of daily living.
Karmakar et al, 2014 [24]	• 19 NeP-DPN (65.7 ± 10)	2 Inertial sensors (ACC and GYR) (GaitMeter™) Freq: not reported	•Shanks	Straight walking test at preferred speed for 50 meters on a flat floor and a 90° turn without rest time.	Step length, step velocity, gait variability	DPN subjects with neuropathic pain receiving pregabalin treatment had increasing variance for both step length and step velocity. No significant differences in durations of time required to walk, step length and step velocity measures were found between timepoints and interventions. The degree of variability in both step length and step velocity significantly increased for subjects receiving pregabalin for comparison of baseline and final visits. The potential relief of NeP using pharmacotherapy may not improve gait dysfunction.

Najafi et al, 2013 [29]	• 12 DPN (60 ± 12) • 8 Healthy controls (60 ± 6)	5 Inertial sensors (ACC and GYR) (LEGSys™, Biosensics LLC) Freq: not reported	•Shanks •Thighs • Lower back	Straight walking test at preferred speed for 7 meters (short distance) and 20 meters (long distance) at two conditions: barefoot and with regular shoes.	Gait initiation velocity, stride velocity, gait variability, average range of motion of ML- and AP- CoM during each stride, double support time, stride time, stride length, number of steps.	Most gait parameters showed alterations in patients with DPN during the barefoot and shoe conditions compared with those in the control group. However, the effect size was usually larger in the long walking distance trials, and none of the observed differences were statistically significant in the short walking distance trials. Gait speed during the gait initiation and gait steady state phases was reduced on average by 15%. Variability was 84% higher in the DPN group. Double support time was more than 20% during the barefoot and shod conditions in those with DPN, suggesting a more altered gait while walking barefoot. The benefit of footwear was significant only during the long walking distance trials.
Lalli et al, 2013 [25]	• 20 DM (60.2 ± 13) • 20 DPN (62.6 ± 9) • 22 NeP-DPN (63.9 ± 9) • 24 Healthy controls (58.8 ± 11)	2 Inertial sensors (ACC and GYR) (GaitMeter™) Freq: not reported	•Shanks	Straight walking test at preferred speed for 50 meters on a flat floor and a 90° turn without rest time.	Gait variability, cadence, step length, step velocity and total duration of walk	No differences were observed among groups in the total duration of walk, step length and step velocity. The degree of variability in both step length and velocity were both significant in participants with NeP-DPN compared to DPN. Participants with NeP-DPN had greater variance in gait when compared to DPN and controls. Also, patients with DPN or DM only were not significantly different from controls with respect to most gait measures utilized. NeP contributes to gait variability, potentially contributing to the risk of falling in DM patients.
Kelly et al, 2013 [30]	• 16 DPN (73 ± 8) • 18 DM (62 ± 8)	5 Inertial sensors (ACC, GYR and MAG) (LEGSys™, Biosensics LLC) Freq: not reported	•Shanks •Thighs • Lower back	Straight walking test at preferred speed for 20 meters on a flat floor	• Gait: stride velocity, stride length, stride time, double support time, gait speed variability, steps required to reach steady-state walking, AP and ML COM sway during walking	Gait performance was relatively worse in participants with DPN compared with DM individuals. However, only steps taken during gait initiation and double-support percentage achieved statistical significance. The DPN and non-DPN groups had almost the same level of concern about falling, suggesting a prevalence in older adults with DM but not a relation with DPN.

Grewal et al, 2013 [31]	• 29 DPN (57 ± 10)	2 Inertial sensors (ACC and GYR) (BalanSens™, BioSensics LLC) Freq: 100 Hz	• 1 Shank • Lower back	Double stance position for 30 s at open and closed eyes (width not specified)	COM sway (AP and ML) and sway area. Postural coordination between the upper and lower body (in the mediolateral and anteroposterior directions)	Significant reduction in center of mass sway after training. A higher postural stability deficit (high body sway) at baseline was associated with higher training gains in postural balance (reduction in center of mass sway). In addition, significant improvement was observed in postural coordination between the ankle and hip joints.
Grewal et al, 2013 [33]	• 16 DPN + DFU (58.3 ± 4) • 15 DPN (54.2 ± 11) • 8 Healthy controls (59.6 ± 6)	A set of Inertial sensors (LEGSys™, Biosensics LLC) (ACC and GYR) Freq: not reported	Not reported	Not reported	Stride velocity, stride length, gait cycle time, double support time, AP- and ML-COM sway area, knee range of motion, gait variability, number of steps and total distance required to achieve gait steady state	During gait initiation, number of steps, knee range of motion and CV stride velocity revealed significant differences among groups. The presence of PNP increases the number of steps required to reach steady state gait by nearly 90% compared to healthy individuals. During steady state gait, double support, COM sway area and CV stride velocity were significantly different between groups. The results demonstrates that neuropathy deteriorates gait, but the presence of foot ulcers does not alter gait parameters further than neuropathy. In addition, patients with foot ulcers demonstrated a better gait compared with DPN patients without ulcers.
Turcot et al, 2012 [41]	• 25 DPN (63.5 ± 7)	3 Inertial sensors (ACC and GYR) (Physilog®, BioAGM). Freq: 200 Hz	• Shanks • Lower back	Double leg stance for 30 s with open and closed eyes (width not specified)	Angular velocity at trunk and ankle levels in two terms: RMS and with cross-correlation function (CCF), to investigate the coordination of human movements in motor control. CFF was calculated between trunk and right ankle, trunk and left ankle, right and left ankle.	The analyses of anterior-posterior angular velocities between the trunk and both ankles showed positive CCFs in the eyes open condition in 23/25 patients and in all patients in the eyes closed condition. It has been demonstrated that the level of PNP was linked to postural strategies and instability during different standing tasks. RMS of the angular velocities at the trunk and ankle levels increases as the task complexity increases. These results highlighted the relation of the level of PNP with postural strategies and instability.

de Bruin et al, 2012 [54]	• 29 DPN (with and without PNP) (61.9 ± 5)	1 accelerometer (DynaPort Mini-Mod, McRoberts BV) (ACC) Freq: not reported	• Lower back	Walking at preferred velocity under two conditions. Single task: walking on the walkway; dual task: walking on the walkway with a counting task. The walkway contained a paved trajectory, cobble stones, and gravel rocks	Step time, step length, velocity, cadence	Significant differences between single versus dual task walking at baseline were identified for all gait parameters. Gait speed, step length, and cadence were significantly decreased under dual tasking, and step duration was significantly increased compared to normal walking. Gait speed, cadence, step duration, and step length under more challenging conditions can be reliably measured in adults with diabetes
Najafi et al, 2010 [35]	• 17 DPN (59.2 ± 8) • 21 Healthy controls (24.4 ± 1)	2 Inertial sensors (ACC, GYR and MAG) (BalanSens™, Biosensics) Freq: not reported	• 1 Shank • Lower back	Double leg stance for 30 s with open (EO) and closed eyes (EC) and feet together, with firm and foam surfaces.	COM sway area, hip and ankle motions	DPN individuals exhibit significantly greater COM sway than healthy subjects during both EO and EC conditions. Sway area was significantly higher than healthy subjects on average by 98%. No significant difference was observed for both ankle and hip sways during EO. At EC, both ankle and hip sways were significantly higher in DPN subjects. Results suggest that postural compensatory strategies during EO condition is significantly better in healthy subjects compared to DPN subjects. During EC condition, although postural control strategy was better in healthy subjects, the observed difference was not significant. It has been shown that PNP significantly affects postural compensatory strategies.

TABLE 2. Summary of the major characteristics of the PD reviews that met the inclusion criteria.

REFERENCE	REVIEW CHARACTERISTICS	NUMBER OF STUDIES INVESTIGATING PD	SAMPLE SIZE (H&Y stage)	SENSORS (number and type)	EXTRACTED PARAMETERS
Morgan et al, 2020 [16]	Analysis of gait during home assessment	11 papers	Almost half of the studies used between 10 and 49 PD participants. 12 studies used fewer than 10 and 8 more than 100 participants.	**45.5%** of the studies used 1 sensor at the lower back; 2 studies used 3 sensors at lower back and feet; 1 paper used 1 sensor on the chest, 1 used 1 sensor on the wrist. 2 papers do not describe the position	Features not specified.
Ghislieri et al, 2019 [22]	Analysis of standing balance	14 papers	From 10 to 58 PD patients (and one study with 104 patients)	The 93% of studies used 1 sensors on the lower back. 1 study used 3 sensors: 1 on the lower back and 2 on lower limbs	Jerk index, sway amplitude, range of acceleration signals, frequency dispersion and centroidal frequency.
Rovini et al, 2018 [17]	Analysis of gait during home assessment	30 papers	Ranging from 1 to 75 PD patients	6 papers **(28.2%)** used 1 sensor: 4 on the waist and 2 on the lower back. 10 **(33.3%)** papers used 2 sensors: 5 on the wrists, 1 on the feet, 3 on the ankles, one on ankle and dominant leg. 6 studies used 3 sensors on the waist and feet. 2 papers used 5 sensors (on wrists, ankles and trunk; on shanks, wrists and sternum). The last 3 papers used more than 6 sensors.	Average time and distance walked, cadence, gait speed, step length, swing time, double support time; stride time and stride time variability. Inter-trial variability, inter-subject variability; inter-task variability. Number of turns per hour, turn angle amplitude, turn duration, turn mean velocity, number of steps per turn, hourly frequency of turning, duration of each turn, number of steps per turn, peak and average rotational turning rate, jerk, variability of these measures throughout the day and week.
Merola et al, 2018 [44]	Analysis of gait and balance	6 papers	From 6 to 40 (and 2 studies with 190 and 139 PD patients)	Not reported	**Gait:** temporal (reaction time, gait cycle duration), spatial (step length, step height) and biomechanical (ankle torque, vertical landing force) variables, and gait strategies (i.e. number of steps, single versus multiple step response). **Balance and postural instability:** trajectory of the center of pressure (COP) and center of mass (COM) misplacement, trunk acceleration and postural sway
Vienne et al, 2017 [20]	General analysis of gait	16 papers	Not reported	11 studies **(68.7%)** described the assessment of PD with 1 sensor at the lower back. one paper used one sensor at one ankle, one at one shank and one at one foot. One paper used 2 sensors (upper and lower back), and one paper utilized 3 sensors at lower back and shanks	Features not specified.

Rovini et al, 2017 [45]	Analysis of wearable sensors on support of PD treatment and diagnosis	80 papers	From 5 to 47 (and 1 study of 75 PD patients)	Not reported	Statistical (e.g., mean, variance, skewness, kurtosis), frequency (e.g., energy, power spectral density, fundamental frequency), and spatiotemporal/kinematic (e.g., stride length, TUG time, stride velocity) features; step or stride segmentation.
Godinho et al, 2016 [14]	Mobile health technology characteristics	76 papers	Not reported	Not reported	ISway measures (jerk, RMS amplitude and mean velocity from the time-domain measures, and centroidal frequency); gait parameters with a high degree of accuracy; total number of walking bouts, the percent of time spent walking, the total number of steps, median walking bout duration, median number of steps, and median cadence per bout. Quality-related sensor derived measures included: frequency measures, regularity measures and the harmonic ratio.
Del Din et al, 2016 [18]	Analysis of gait during home assessment	19 papers	From 2 to 169 PD participants (and one study of 467 patients)	9 studies **(47.3%)** used 1 sensor on lower back; 3 used 2 sensors on thighs; 2 papers used 2 sensors on feet; 1 on both shanks and 1 used 1 sensor on the chest; the other papers used more than 4 sensors.	Number of walking bouts, walking duration, total number of steps, median number of steps per bout, bout duration, cadence, step and stride regularity, frequency domain measures (harmonic ratio, amplitude, slope and width of dominant frequency), step duration, step symmetry, acceleration range and dynamic stability
Oung et al, 2015 [43]	Assessment of motor disorders in PD	Not reported	Not reported	Not reported	Step frequency, stride length, entropy and arm swing
Hubble et al, 2015 [23]	Analysis of standing balance and walking stability	26 papers	From 5 to 67 PD patients	20 studies **(76.9%) used** 1 sensor on the lower back (sacrum/ L3/ L4/ L5); 2 studies used 2 sensors on the shanks; 2 studies used 1 sensor on sternum/chest; 1 study utilized one sensor on the wrist; and another one on the lateral side of the pelvis.	Sway velocity (23% of studies), RMS accelerations (19% of studies) and jerk (19% of studies). Harmonic ratio (31% of studies) and stride time variability (27% of studies).
Steins et al, 2014 [43]	Assessment of functional activities with wearable devices	6 papers	Not reported	Not reported	Stride length, stride velocity, cadence, and turning velocity

Maetzler et al, 2013 [21]	Quantitative objective assessment of gait and balance	16 papers	Not reported	**Gait**: 4 papers used one sensor on the lower back **(44.4%**). 2 papers utilized 1 sensor on the shank and 2 papers 2 sensors on both feet. 1 paper used 1 sensor on the forearm and two studies used more than 5 sensors. **Balance**: 5 papers used 1 sensor on lower back **(100%)**.	**Gait:** Phase coordination index of gait cycle; stride length; frequency-based measures of gait (harmonic ratio, amplitude, slope and width of dominant frequency); cadence, step time variability; peak trunk horizontal velocity, turning duration, turn-to-sit duration; time- and amplitude-based measures of sit-to-stand and stand-to-sit; peak trunk rotation velocity and rotation range of motion, turning velocity; Walk peak roll velocity, total turning duration, turn peak yaw and roll velocity. **Balance**: Velocity, jerk, acceleration, frequency-based measures; displacement, velocity; Peak trunk acceleration during anticipatory postural adjustments towards the stance leg; Hilbert-Huang transformation of postural parameters
Horak et al, 2013 [42]	Biomarkers of gait and balance	Not reported	Not reported	Not reported	**Gait**: Stride Time Variability, double support time, peak arm velocity, trunk rotation, gait velocity, cadence, stride length. Balance: Postural sway (area, velocity, frequency) and jerk.

Note: ACC: Accelerometer; **AP**: Anterior-posterior; **CIDN:** Chronic inflammatory demyelinating polyneuropathy; **CIPN**: Chemotherapy-induced peripheral neuropathy; **COG**: Center of gravity; **COM**: Center of mass; **DFU**: Diabetic foot ulcer; **DM**: Diabetes Mellitus; **DPN**: Diabetic peripheral neuropathy; **Freq**: sample frequency; **GYR**: Gyroscope; **MAG**: Magnetometer; **ML**: Medio-lateral; **NeP-DPN**: Neuropathic pain diabetic neuropathy; **PNP-LL**: Peripheral neuropathy of the lower limbs; **TUG**: Timed Up and Go test.

DISCUSSION

We conducted this systematic review to establish the most appropriate approach targeting the number and placement of wearables and most clinically relevant outcomes to assess PNP associated gait and balance dysfunction in PD patients. We identified the main findings and highlighted general conclusions and suggestions for further study protocols based on (1) how often the parameter is assessed, or how often the sensor is placed on a specific location, (2) the statistical significance of the parameter in the included studies (compared to a control group), (3) the clinical relevance of the parameter in relation to the main scope of the included studies. To our best knowledge, this is the first review to evaluate the existing evidence on PNP-PD.

The research on wearable health technology to address PNP characterization is lacking, as demonstrated by the small number of studies found according to the inclusion criteria of this review. Almost all the studies included patients with diabetes mellitus (DM) or patients with cancer undergoing chemotherapy. Both conditions have severe consequences on the peripheral nervous system and affect somatosensory function. In particular, diabetic peripheral neuropathy (DPN) affects up to half of the population with diabetes [32] and chemotherapy-induced PNP (CIPN) afflicts up to 40% of patients suffering from cancer [33]. As PNP is most probably a PD-associated symptom, we investigated the main PNP and PD motor characteristics to guide future studies using wearable technology to consider this phenotype in PD. All studies included in this review aimed to investigate both PNP motor deficits and its contribution to (increased) risk of falling and PNP sensory deficits that lead to inadequate proprioceptive feedback, affecting stability during standing and walking. Therefore, given the impact of sensory nervous system in both gait and balance motor activities, we analyzed both domains, gait and balance.

Gait and Walking Stability

Numerous abnormalities, including sensory loss (impaired vibration, protective sensation), decreased lower-extremity strength, and alterations in the central nervous system, contribute to impaired gait in PNP [57].

Our literature search showed that studies investigated mainly gait aspects in PNP patients: Eleven studies examined gait as major primary outcome, while only five papers assessed balance and postural stability (in addition to gait assessment). An explanation for the preference of gait assessment over balance and posture assessment may be the fact that, especially in DPN, the numbness of the feet is considered a major risk factor for increased deterioration in gait function and walking stability [31]. Moreover, footwear that improves gait has been shown to improve quality of life in PNP patients.

In terms of sensor placement, the amount and the exact position of sensors should consider expected outcomes, practicality, and ease in reproducing the sensor placement [25]. In the selected studies, we found neither a consensus on the position nor on the number of sensors used to investigate gait: Esser et al. [17] showed that a single sensor has the potential to discriminate DPN patients from controls, but it was generally preferred to place sensors on both lower limbs (on the shanks or thighs or both) together with an extra sensor on the lower back. A setup of more than one sensor was preferred in more than 70% of the selected studies, in contrast to PD setups that prefer a smaller number of sensors, usually involving one sensor on the lower back [58]. Generally, gait assessment in PD is performed with one wearable located as close as possible to the COM (i.e., on the lower back) or on one lower limb. This solution is adopted for two reasons: Firstly, this position can track a large amount of body movements (including gait asymmetry and variability, if the sensor is placed on the lower back) [59] and, secondly, it facilitates and simplifies the use of wearables, reducing the intra- and inter-operator variability.

We believe that the discrepancy between PNP and PD sensors' setups could be attributable to the expected outcomes and intrinsic characteristics of both pathologies: In PNP the assessment of gait focuses more on variability, step width, and clearance of the feet and, thus, it makes sense to position sensors on both feet. In contrast, gait evaluation in PD relates more to "whole body" or axial movements [60].

Nowadays, a plethora of physical capability assessments and associated algorithms have been developed for the use of one sensor [59], encouraging the simplification of assessment in PD. Since in specific pathological situations the use of sensors placed on both legs is recommended so that data from both sides can be merged [61] and spatial parameters (such as step length, width, and height) are generally more accurate when calculated with a foot or shank sensors, we support the use of more than one sensor for this specific subset of PNP-PD patients (on the lower back and on the lower limbs) to assess gait.

Spatiotemporal parameters extracted in the selected manuscripts were not always statistically significant in the analysis of PNP compared to healthy participants' gait. Overall, these results confirmed that, in PNP, the loss of sensation and the inability of the

neuromuscular control system to respond to a challenging environment during walking is stronger when attention is reduced [62]. Gait speed and gait variability [29–31,34] demonstrated to have a clear association with falling, resulting in relevant parameters to consider when evaluating PNP gait. This is also corroborated by previous literature showing a significant decrease in quality of spatiotemporal parameters, especially for DPN patients [63]. Lastly, the number of steps and distance to reach steady-state gait in the analysis of gait initiation were found to be an important component to investigate risk of falls in people with PNP [35,37,38]: It has been shown that PNP patients take more and slower steps and a longer distance to reach steady-state gait compared to controls. This is due to a decreased somatosensory function, which directly a ects performance in the gait initiation phase, increasing unbalance postural transitions and, consequently, the risk of falls. Spatiotemporal and frequency-based measures can discriminate PD patients from controls and may also have some potential as surrogate markers for quality of life and disease severity in PD patients [52].

In order to gather all the aspects on gait deficits in PD and to reflect a more true-to-life condition, a large amount of papers on PD motor assessment included functional tests to assess various multifactorial aspects other than gait [53]. An example is the use of the instrumented TUG (iTUG) test, which provides an “overview” of functional mobility by assessing sit-to-stand, straight walking, turning, and stand-to-sit movements [49]. The use of such tools have been shown to be effective to assess gait in PD [64], while for PNP it was only used in a minority of the papers appraised in this review (N = 3).

In addition, monitoring patients in a daily-living environment and over continuous time periods can make the assessment feasible and ecological. This approach is widely used in PD [23,65], while for PNP only one of the selected papers used monitoring at home to assess gait performances [18].

Balance and Postural Stability

Postural control depends on sensory feedback, which includes visual, vestibular, and somatosensory systems. To maintain balance, the central integration of proprioceptive information from the legs with other sensory information is necessary [57]. Individuals with PNP experience balance impairments during gait and standing position, due to absent sensory responses from the lower limbs. This loss in sensory input generally causes instability in trunk sway in people with PNP, even though balance corrections following perturbations to stance are still initiated [24].

Our literature search revealed nine of the included manuscripts investigating static balance and postural stability in PNP and four other studies analyzing both gait and balance abnormalities.

Static balance tasks comprehended a variety of conditions whose general aim was to detect minimal significant perturbations. The most usual adopted strategy was to reduce the support base, asking the subjects to stand still with feet together (which was the assessment protocol in 70% of the selected papers). This approach was widely used because it is easily understandable, repeatable, and can be simply applied to older patients. Other strategies to challenge balance control, such as tandem or semi-tandem positions or one-legged stance, were rarely used because they are relatively difficult to handle for this type of patient (Table 1).

Only 15.5% of the studies [24,44] asked participants to keep feet apart (usually shoulder's width or, more specifically, 10 cm between heels and 15 cm between halluces) during assessment, which is in line with a study by McIlroy and Maki [66], who recommended to avoid 'unnatural' or 'uncomfortable' foot positions in favor of a preferred foot placement. The strategy of open and closed eyes and the use of foams were adopted in order to reduce the remaining contribution of lower leg proprioceptive feedback to balance control and to understand the level of visual cueing in PNP patients. Four studies performed balance tasks barefoot [24,43,46,48], an interesting approach that could be applied to emphasize PNP impairments, even if not always applicable because of neuropathic complications (i.e., diabetic foot ulcerations) [67].

In PD, a standard feet position during stance tests is not fully established [27]. When it is preferred to keep the feet apart, because it is a more ecological condition, the performances can be biased by the subjective selection of the base of support. This can lead to contradictory findings due to methodological differences between subjects and studies. To avoid discrepancies, Hubble et al. [28] recommended to stand with eyes open and feet of maximum 10 cm apart during stance tests.

Several ways exist for estimating postural sway. An important rule to consider is to place at least one inertial sensor at the lower back, often the best position to monitor the COM [43], to examine both PNP- and PD-related deficits. A single accelerometer worn on the lower back has been validated to assess balance characteristics [68], but this approach may be not appropriate for assessing postural sway, for example, during large sway fluctuations or reaching task movements [43]. To overcome this defect, using more than one sensor, especially on the lower limbs, is recommended. This is also confirmed by the included studies: Ten of 13 papers used more than only the sensor on the lower back (Table 1). Moreover, this is also confirmed in PD assessments: One sensor on the lower back was used to perform posturographic examination, while additional sensors on the lower limbs were preferred to assess (further) postural strategies [27].

Regarding the relevant features for balance and postural stability, interesting conclusions can be made from the included studies of PNP. First of all, compared to healthy controls,

COM-AP sway amplitude seems to be associated with the presence of neuropathy symptoms [44,47]. This is in line with evidence from literature: Higher AP sway may be associated with PNP as a result of an increased sway at the hip joint [69]. In fact, healthy individuals rely on the ankle joint to control sway (ankle strategy), while PNP patients predominantly showed a hip strategy, to benefit from more accurate proprioceptive information from receptors at the hips [70].

A second notable result is that COM-ML sway amplitudes are obviously a good predictor of falls. It has been shown that ML sway was associated with falls in PNP patients [42,44]. These data are consistent with other populations, such as elderly [71].

Clinical trials did not find significant differences in postural sway before and after treatment between intervention and control groups. However, the most promising parameter may be ankle sway: this parameter showed the highest effect size (Cohen's $d = 0.76$; $p = 0.001$) after plantar electrical stimulation [39].

In PD, postural sway in both AP and ML directions was also the most analyzed feature during stance tests [53]. Other relevant parameters of postural stability are jerk index, the range of acceleration signals, frequency dispersion, and centroidal frequency [27].

Overall, AP, ML, and total sway frequencies need to be taken into consideration when investigating postural stability in PNP [46] and PD, using both open- and closed-eyes tasks and static and dynamic balance tests [24], in addition to hip and ankle sway (for both hip and ankle strategies). The last was shown to be greater also in CIPN patients during both eyes-open and -closed conditions, suggesting a pronounced visual dependency of PNP for ankle stability [56]. A final consideration to point out is the feasibility of wearables in assessing motor symptoms. Among the included papers on PNP, IMUs' feasibility and accuracy were investigated by Najafi et al. [43], who compared balance features with center of pressure (COP) measures from a standard pressure platform in a group of healthy subjects and in a group of PNP patients. Results suggested a relatively high correlation ($r = 0.92$) between the two measurements during all the study conditions, and the same IMUs' protocol was then used and repeated in other further studies from the same group [18,56]. In addition, the same IMU measures were compared to clinical scores during different conditions (open-eyes and closed-eyes conditions). With regard to PD, IMUs' accuracy and feasibility were pointed out in the work by Oung et al. [50], who compared this technique with video recording and clinical evaluation (i.e., Unified Parkinson's Disease Rating Scale – UPDRS scores). Sensitivity and validity of IMUs were also confirmed in the review by Godinho et al. [16]: Reliability was investigated comparing IMUs' sway with force-plate measures, and test-retest reliability were also confirmed by clinical balance tests. For both pathologies (PNP and PD), we found no information on accuracy and feasibility based on sensor location.

PNP Motor Assessment with Other Tools than Wearables

Clinical scales and complex approaches are noteworthy in the evaluation of PNP functional disabilities, although these tools present disadvantages: They are time-consuming and require specific expertise. In addition, complex tools are reserved only for clinical settings due to their high cost and complexity of technology and can capture only a few steps and often do not represent the full gait complexity. An overview of the main clinical scales and these complex systems is provided in the following paragraphs.

Gait Assessment

Clinical scales represent reliable and valid measures of disease characterization and monitoring. Worth mentioning in the evaluation of PNP gait disturbances are the functional gait assessment scale, which effectively classifies fall risk and predicts unexplained falls [72], and the Dynamic Gait Index, assessing the ability to adapt gait to complex tasks and walking stability [73]. For their efficacy and sensitivity, these clinical scales are often chosen as primary outcomes in intervention studies. More complex equipment was also used to evaluate gait in PNP. The 3D optical motion capture systems measure the position and orientation of corporal segments in space [74] and provide a large amount of gait characteristics that can be investigated. Optical motion capture systems are often combined with force plates: mechanical sensing apparatus designed to measure the ground reaction forces and moments involved in the human movement [75]. The vast majority of studies on gait assessment in individuals suffering from PNP used optical motion capture systems, force plates, or a combination of the two (56.7% of the included papers). Particularly, foot and foot joints were relevant targets in the investigation of DPN. This is due to the fact that PNP is one of the key factors in the pathogenesis of diabetic foot and its chronic complications [76]. Hip abductors' range of motion or hip angles, knee flexion, ankle joint dorsiflexion, and metatarso-phalangeal flexion-extension were the focus of investigation of gait patterns in PNP with motion capture analysis [76–80]. Differences were found in spatiotemporal parameters during walking on smooth and uneven surfaces in DPN [81], while a significant increase was found in toe clearance [78,82] and step width [76,83] of PNP patients compared to controls. Other relevant features analyzed were foot rotation on the sagittal plane, knee and ankle strength [84], dorsal and plantar flexors strength [85], dynamic plantar pressure at the forefoot [86], and peak forces of ankle (flexors, extensors, and evertors) [77]. Another frequent tool (in 19.4% of the included papers) in the examination of PNP gait was the use of electronic walkways. These electronic walkways are pressure-sensitive carpets (the most used was the GAITRite® system), a computerized

walkway system for the quantification of spatiotemporal gait parameters. They are portable and embedded with pressure sensors that detect a series of footfalls [87]. Electronic walkways were used for the analysis of gait in PNP subjects to study treatment effects [88], to characterize PNP global gait [89], to investigate the functional impairment in daily activities [90], to study cognitive deterioration during dual-task condition [91] and to analyze gait patterns at different locomotion speeds [92].

Balance and Postural Stability

For the examination of balance performances in PNP, the Berg Balance Scale (BBS) was the most used clinical scale [73,93–99]. BBS is a standard clinical measure to assess static balance impairments and a robust method to study postural control [100]. The Tinetti Balance scale (TBS) is another valid clinical scale to measure balance: Monti Bragadin et al. [99] demonstrated the importance of both TBS and BBS tests in the evaluation of disability in PNP and, in particular, in identifying those patients who present a substantial risk of falling. The Fullerton Advance Balance test (FAB) [101-103] is being increasingly utilized because of its capacity to assess postural control among higher functioning independent older adults [104]. Contrary to the BBS, FAB test examines both static and dynamic postural control, sensory reception, and integration and incorporates a secondary task [100]. A few studies utilized the Romberg test to assess postural stability with simple scoring 'pass or fail' [19,105]. Participants were classified as having dysfunctional balance if they failed any of the four Romberg test conditions. Although quick and simple, this method cannot define postural stability impairments with accuracy. With respect to other approaches, most studies have employed force plates in the evaluation of postural stability (71.4% of the papers included in the narrative search). Force platforms measured the COM projections over the base of support and recorded postural stability in two ways, with static and dynamic posturography. The dynamic approach analyzes postural reactions in response to a translation of the support surface, to the visual surrounding, or both [106]. Static balance assessment was more adopted compared to dynamic posturography (in 64.2% of the included papers) in the investigation of PNP. Static posturography with force plates was used to evaluate the effect of a rocker outsole shoe on postural stability [107] and of a new insole design [108] in individuals with DNP. Manor et al. [109] and Alsubiheen et al. [110] used static balance assessment with force plates to examine the effects of Tai-Chi on standing COP dynamics in adults with PNP, resulting in an increased complexity of standing dynamics and significant improvement after intervention. Force platforms were used to quantify differences in postural stability: to assess the effect of intervention on stability in CIPN survivors [96,111], the impact of a sensorimotor exercise program [103,112], and the influence of a balance and endurance training, which resulted in an

improvement in sway path [113]. Changes in body sway were also compared between DNP and Charcot-Marie Tooth subjects, indicating more impaired static control of balance in the DNP group, possibly due to small and large afferent fibers' involvement [114]. Static balance assessments also allowed evaluating postural control and fall incidence in PNP [115], to assess postural stability in the PNP population on either firm or foam surfaces [116], and to differentiate between PNP and healthy controls [117]. Moreover, static balance was also examined without the use of force plates in five studies (17.8%). McCary et al. [118] used a swaymeter (Neuroscience Research Australia, Sydney) to quantify postural sway pre- and post-rehabilitation in people with CIPN. In another study, sway amplitude and velocity were analyzed through a head and hip electromagnetic tracker [119]. Finally, baropodometric platforms were used in three studies [97,120,121]: These tools use the load and the plantar pressure on the mat to define footprint shape and assess foot deformities and barefoot plantar pressures. Dynamic posturography was chosen in the 28.5% of the studies and comprehended the sensory organization test (SOT). During the SOT, subjects are instructed to stand still and maintain balance using the visual, vestibular, and proprioceptive systems. The SOT evaluates patients' ability to effectively use the three sensory systems to maintain postural stability. In PNP, dynamic balance tests with force platforms were used to evaluate the altered sensory organization during stance [122] and postural sway reactions [123] in CIPN patients. This approach was also chosen to assess standing postural reactions in demyelinating PNP [124] and the effects of PNP in detecting short postural perturbations [125]. A study by Razzak and Hussein [126] highlighted a greater visual dependence in DNP patients faced with postural challenging situations, while Rao and Aruin [127] suggested that auxiliary sensory cues improved automatic postural responses.

In conclusion, wearable health technology is increasingly becoming an attractive alternative to conventional assessment tools to assess PD, PNP, and PD-PNP patients in clinical routine management and in clinical trials. These novel technologies have greater applicability especially for the assessment of daily life activities and, finally, are cheaper and less complex compared to conventional, lab-based equipment.

CONCLUSIONS

We consider the use of wearable health technology for the assessment of PNP in PD of great advantage compared to clinical scales and conventional, lab-based assessment tools, as the former allow for more consistent and reliable results.

The following suggestions may help assessing this cohort (Figure 4):

- A combination of at least two sensors (one on lower back and one on at least one lower limb) may help gathering both PNP- and PD-specific features during gait and balance testing.
- Concerning parameters to analyze, particular attention should be given to gait speed, stride length, and gait variability. Gait variability may be particularly relevant for PNP-induced gait changes. Dual tasking assessments and irregular trajectories may unveil PNP-related gait deficits that are not visible during nonchallenging, single tasking walking conditions.
- Functional mobility tests (TUG test, functional reach test) can provide a comprehensive overview of function and mobility in PD patients with and without PNP.
- Balance tasks should include double leg stance with open- and with closed-eyes conditions.
- Total sway amplitude and AP and ML sway directions may be the most promising balance parameters to differentiate between PD and PD-PNP.

Overall, these suggestions may help to accurately stratify and monitor PD- and PNP-associated functional deficits of gait and balance and target personalized treatments and strategies to prevent falls. This could have an impact on the diagnosis and clinical approach of this subset of patients in particular on the aged population in general.

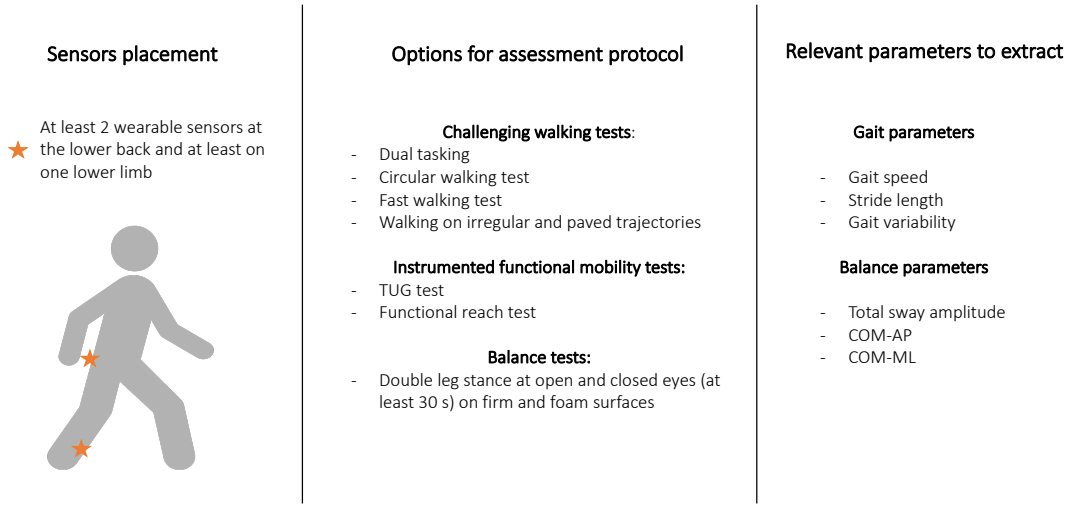

Figure 4. Suggestions for the motor assessment of PNP-PD cohort with wearable health technology.

Appendix

Search query

1) Pubmed

-PN+wearable with MeSH

((Peripheral Nervous System Diseases[Mesh]) AND (wearable sensor*[Title/Abstract] OR wearable [Title/Abstract] OR mobile health technology[Title/Abstract] OR technology assessment[Title/Abstract] OR body-worn sensor*[Title/Abstract] OR portable device[Title/Abstract] OR inertial sensor[Title/Abstract] OR inertial measurement unit[Title/Abstract] OR acceleromet*[Title/Abstract] OR gyroscope[Title/Abstract] OR angular velocity[Title/Abstract] OR acceleration[Title/Abstract]) AND(mobility[Title/Abstract] OR gait[Title/Abstract] OR balance[Title/Abstract] OR postural balance[Title/Abstract] OR postural stability[Title/Abstract] OR postural strategies[Title/Abstract]))

-PD+wearable with MeSH terms

((Parkinson Disease[Mesh]) AND (wearable sensor*[Title/Abstract] OR wearable [Title/Abstract] OR mobile health technology[Title/Abstract] OR technology assessment[Title/Abstract] OR body-worn sensor*[Title/Abstract] OR inertial sensor[Title/Abstract] OR inertial measurement unit[Title/Abstract] OR acceleromet*[Title/Abstract] OR gyroscope[Title/Abstract]) AND (mobility[Title/Abstract] OR gait[Title/Abstract] OR balance[Title/Abstract] OR postural balance[Title/Abstract])).

2) Scopus database

-PN+wearables

TITLE-ABS-KEY ("peripheral neuropath*") OR TITLE-ABS KEY (polineuropath*) OR TITLE-ABS-KEY ("small fiber neuropathy") AND TITLE ABS-KEY ("wearable sensor*") OR TITLE-ABS-KEY (wearable*) OR TITLE-ABS KEY ("mobile health technolog*") OR TITLE-ABS-KEY ("technology assessment") OR TITLE-ABS-KEY ("body-worn sensor*") OR TITLE-ABS KEY ("inertial sensor*") OR TITLE-ABS-KEY ("inertial measurement unit*") OR TITLE-ABS-KEY (accelerometer*) OR TITLE-ABS KEY (gyroscope*) OR TITLE-ABS-KEY (angular velocity) OR TITLE-ABS KEY (acceleration) AND TITLE-ABS-KEY (mobility) OR TITLE-ABS KEY (gait) OR TITLE-ABS KEY (balance) OR TITLE-ABS-KEY ("postural balance") OR TITLE-ABS-KEY ("postural stability") OR TITLE-ABS-KEY ("postural strategies")

-PD+wearables

TITLE-ABS-KEY (“parkinson’s disease”) OR TITLE-ABS-KEY (“parkinson disease”) OR TITLE-ABS-KEY (Parkinson*) AND TITLE-ABS-KEY (“wearable sensor*”) OR TITLE-ABS-KEY (wearable*) OR TITLE-ABS-KEY (“mobile health technolog*”) OR TITLE-ABS-KEY (“technology assessment”) OR TITLE-ABS-KEY (“body-worn sensor*”) OR TITLE-ABS-KEY (“inertial sensor*”) OR TITLE-ABS-KEY (“inertial measurement unit*”) OR TITLE-ABS-KEY (accelerometer*) OR TITLE-ABS-KEY (gyroscope*) AND TITLE-ABS-KEY (mobility) OR TITLE-ABS-KEY (gait) OR TITLE-ABS-KEY (balance) OR TITLE-ABS-KEY (“postural balance”)

2) Web of Science database

-PN+wearables

TS=(“peripheral neuropath*” OR polineuropath* OR “small fiber neuropathy”) AND TS=(“wearable sensor*” OR wearable* OR “mobile health technolog*” OR “technology assessment” OR “body-worn sensor*” OR “inertial sensor*” OR “inertial measurement unit*” OR accelerometer* OR gyroscope* OR “angular velocity” OR acceleration) AND TS=(mobility OR gait OR balance OR “postural balance” OR “postural stability” OR “postural strategies”)

-PD+wearables

TS=(“parkinson’s disease” OR “parkinson disease” OR Parkinson*) AND TS=(“wearable sensor*” OR wearable* OR “mobile health technolog*” OR “technology assessment” OR “body-worn sensor*” OR “inertial sensor*” OR “inertial measurement unit*” OR accelerometer* OR gyroscope*) AND TS=(mobility OR gait OR balance OR “postural balance”)

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CHAPTERS 5.

PERIPHERAL NEUROPATHY IN PARKINSON'S DISEASE: PREVALENCE AND FUNCTIONAL IMPACT ON GAIT AND BALANCE

This Chapter was adapted from the published work:

Corrà MF, Vila-Chã N, Sardoreira A, Hansen C, Sousa AP, Reis I, Sambayeta F, Damásio J, Calejo M, Schicketmueller A, Laranjinha I, Salgado P, Taipa R, Magalhães R, Correia M, Maetzler W, Maia LF. Peripheral neuropathy in Parkinson's disease: prevalence and functional impact on gait and balance (Open Access Journal).

ABSTRACT: Peripheral neuropathy (PNP) is a common problem in patients with Parkinson's disease (PD). PNP prevalence in PD varies between 4.8% - 55%, compared to 9 % in the general population. It remains unclear whether PNP leads to decreased motor performance in PD, resulting in impaired mobility and increased balance deficits. We aimed to determine the prevalence and type of PNP in PD patients, and evaluate its functional impact on gait and balance.

A cohort of consecutive PD patients assessed by Movement Disorders' specialists based on the UK Brain Bank criteria underwent clinical, neurophysiological (nerve conduction studies and Quantitative Sensory Testing) and neuropathological (Intraepidermal nerve fiber density in skin biopsies' punches) evaluation, to characterize PNP type and etiology. Gait and balance were characterized using wearable health-technology at OFF and ON medication states and the main parameters were extracted using validated algorithms.

A total of 99 PD participants with a mean age of 67.2 (±10) years-old and mean disease duration of 6.5 (±5) years were assessed. Based on a comprehensive clinical, neurophysiological and neuropathological evaluation we found that 40.4 % of PD patients presented PNP, with a predominance of small fiber neuropathy (70 % of the PD-PNP group). At OFF state, the presence of PNP was significantly associated with shorter stride length ($p=0.029$), slower gait speed ($p=0.005$) and smaller toe-off angles ($p=0.002$) during straight walking; significantly slower speed ($p=0.019$) and smaller toe-off angles ($p=0.007$) were also observed during circular walking. At ON state, the above effects remained, albeit moderately reduced. With regard to balance, significant differences between PD without PNP (PD-noPNP) and PD-PNP were observed at OFF medication state during stance with closed eyes on a foam surface. At ON states, these differences were no longer observable. We showed that PNP is common in PD, and influences gait and balance parameters, as measured with mobile health-technology. Our study supports that PNP recognition and

directed treatment should be pursued in order to improve PD patient's gait and minimize balance related disability, targeting individualized medical care.

Keywords: Parkinson's disease; peripheral neuropathy; wearable health-technology; functional impact.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder leading to significant disability and decreased quality of life. With disease progression, motor impairment represents a considerable burden, and gait and balance deficits progressively increase the risk of falls and the managing of daily-life activities (1, 2). Apart from the hallmark motor symptoms, PD is considered as a multi-systemic disorder of the nervous system, and non motor symptoms have received increasing interest in recent years (3). Among the main features of PD, a growing number of studies assessing peripheral nerve pathology have recognized the increased prevalence of peripheral neuropathy (PNP) in the PD population (4-6).

PNP is a disorder of the peripheral nervous system (PNS) whose main manifestations are postural instability, loss of peripheral sensation, weakness and pain. PNP usually exhibits a distal-proximal gradient, affecting first the feet (7). PNP can be classified into large fiber neuropathy (FN) and small fiber neuropathy (FN), affecting predominantly myelinated and unmyelinated fibers, respectively (8).

Large fiber Neuropathy (large-FN) in PNP is diagnosed via the assessment of nerve conduction velocity and amplitude of the electric signal. Small FN is diagnosed through a composite evaluation including the assessment of neurological signs and symptoms, specific neurophysiological tests such as the quantitative sensory testing (QST) and nerve fiber quantitative characterization (9).

This diagnostic approach must be systematic to increase specificity (9). PNP was initially considered only in rare genetic forms of PD (10, 11), but a significant number of PD patients have shown PNP, first in case-series and later in multi-centric studies (12-14).

The prevalence of PNP in PD varies depending on the diagnostic methods used, and has been shown to be present in up to 55 % of PD patients (5, 7, 12, 13), compared to 8-9 % in the general population with similar age (15). The association of PNP with PD has different explanations: i) it may be linked to Levodopa (L-dopa) intake (7), proved by a higher prevalence of PNP in patients treated with L-dopa compared to those not treated with L dopa (16), and by a higher prevalence of PNP in patients receiving duodopa or L-dopa intestinal gel compared to oral L-dopa (16, 17); ii) it may also be an intrinsic feature of PD, related to loss of small nerve fibers due to, e.g., a-synuclein aggregates (main component

of Lewy bodies) not only in the basal ganglia, but also in peripheral nerve structures (5, 18) iii) concomitant diseases, such as metabolic diseases, autoimmune disorders or infections (9, 19).

Importantly, PNP in PD could increase the disability of those affected, leading to additional motor dysfunction (14), higher risk of falls and injuries (20), and worsening of the global functional mobility. Mobility can be evaluated via wearable health technology, which provides objective and quantitative measures of movements, with a precise estimation of spatio-temporal parameters, allowing high sensitivity, accuracy and reproducibility (21). In fact, the use of wearable health technology for the assessment of PNP in PD may provide complementary information to clinical and conventional lab-based assessment tools (22). In order to clarify if PNP has a functional impact on gait and balance in PD (22), we specifically aimed to (1) investigate prevalence and types of PNP in PD with a comprehensive assessment of clinical, neurophysiological and neuropathological evaluation; (2) determine whether PNP contributes to impaired mobility in PD using wearable health technology.

METHODS

Study participants and PD assessment

We conducted a cross-sectional study with consecutive PD participants diagnosed by a Movement Disorders' specialist from Centro Hospitalar Universitário do Porto (CHUPorto) based on the UK Brain Bank criteria (23), and attending the CHUPorto Movement Disorders' outpatient clinic. The possibility of cohort enrichment for the purpose of groups comparison from Movement Disorders' specialists not involved in this study was also prespecified. Demographic and disease specific variables (disease duration, information on daily dopaminergic intake (LEDD) (24) and number of falls, the complete UPDRS scale at both OFF and ON states) were collected. In addition, cognitive tests (DRS), non-motor symptoms scale (NMSS), quality of life questionnaire (PDQ-39) were performed. The study was approved by the institutional review board of CHUPorto (N/REF 2018.087(076-DEFI/076-CES)) and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects before participation.

PNP investigation

Clinical assessment

The presence and severity of clinical signs and symptoms characteristics for PNP were evaluated with the use of two scales. The Neuropathy Impairment Score for Lower Limbs

(NIS-LL) included the measurement of muscle strength, tendon reflexes and sensation of touch pressure, vibration and joint position at the lower limbs (25). This scale is age-adjusted such that decreased ankle reflexes were considered normal or absent over the age of 70. Participants with a NIS-LL of 3-5 points for the reflexes and sensory parts were considered having mild neuropathy signs, those achieving 6-8 points as having medium neuropathy signs, and those achieving above 9-10 as having severe neuropathy signs (26). The modified Toronto Clinical Neuropathy Score (mTCNS) was used to collect information about participants' perception of discomfort and neuropathic symptoms (namely foot pain, numbness, tingling and weakness) (27). Participants with a total score of $\geq$ 6 points were considered to have symptoms of PNP (28).

Neurophysiological assessment

Sensory and motor nerve conduction studies (NCS) were performed using surface recording electrodes with standard placement. The evaluation was performed in the lower limbs (sural sensory, medial plantar, peroneal motor and tibial motor NCS, including F-waves). If any of the previous action potentials were below the normative values, the evaluation extended to the upper limbs (ulnar and median sensory and motor NCS and radial sensory NCS). If a response was absent for any of the above-mentioned nerves (sensory or motor), a NCS of the contralateral nerve was performed. In order to assess small nerve fibers, QST examination using the CASE IV system was used to determine the thermal (cold) and heat-pain thresholds through a multimodal approach (29, 30). Stimuli were tested on the lower limb (dorsal foot), usually in the same limb as the nerve conduction studies were performed. The testing algorithms were the 4, 2, and 1 stepping method for cold thresholds and the non-repeating ascending with null stimuli for heat-pain thresholds. Normative data from the CASE IV system were used. If any of the tests showed altered results, i.e. above the 97th percentile, the upper limb (dorsal hand) of the same side was also evaluated (31).

Neuropathological assessment

Skin specimens were obtained from all participants not taking anticoagulant medication (N=87) with a disposable 5-mm circular punch under sterile technique after topical anesthesia. The anatomical sites of skin biopsies were the lateral side of the distal leg (10 cm above the malleolus) and the proximal thigh (20 cm below the greater trochanter). Fixation and incubation of specimens were performed as previously reported (32). Immunohistochemical labeling was performed on 50-µm frozen sections using rabbit polyclonal protein-gene-product (PGP9.5) antibody (Zytomed systems, Berlin, Germany; 1:250), and appropriate Cyanine 3 (Jackson ImmunoResearch Laboratories, West Grove,

PA, USA; 1:50) as fluorescent secondary antibody. Density was calculated as the number of intraepidermal nerve fibers (IENF) per length of section (IENF/mm). All the tissue sections were analyzed using Nikon Eclipse E400 fluorescence microscope at 40X magnification. Two criteria were considered to quantify the presence of nerve fiber loss: the normative distal cut-off values reported in literature, stratified by age and sex (33) and the gradient between proximal and distal values of IENF (60% or less IENF in the distal probe, compared to the proximal probe, were considered pathologic) (34). To investigate the possible link between peripheral nerve fiber loss and PD pathology, phospho-α-synuclein detection was performed with the same skin specimens to determine potentially PD-driven pathology. 20-µm serial cryosections were cut and double-immunofluorescence labeling was performed using PGP9.5 and anti phospho-synuclein (Biolegend, San Diego, CA, USA; 1:500) and appropriate Cy3 and Alexa Fluor488 (Biolegend, San Diego, CA, USA; 1:1000)-conjugated secondary antibodies. Biopsies were evaluated at the same fluorescence microscope and classified as positive if at least one dermal nerve fiber phospho-α-synuclein-immunoreactive in the entire tissue section (18).

Blood PNP panel

A case-by-case laboratory work-up was performed by a PNP specialist to screen for PNP etiology. Complete blood count, immunoglobulins, T4L and TSH, fasting glucose, glucose tolerance and hemoglobin HbA1C, electrolytes, erythrocyte sedimentation rate, HIV, hepatitis B and C virus serology, antinuclear antibodies, creatinine, blood urea, liver function tests (ALT, AST, ALP, bilirubin, LD, GGT) vitamin B6 and B12 levels, methylmalonic acid (MMA) homocysteine (Hcy) and folic acid levels were conducted (9). We first checked vitamin B6 and B12 deficiency, and vitamin B6 toxicity (41, 42). Vitamin B12 deficiency was considered if vitamin B12 levels were below 191 [pg/L], or vitamin B12 levels were < 500 pg/L, and methylmalonic acid (MMA) and/or homocysteine (Hcy) were above cut-off (43).

Diagnostic criteria for PNP

Participants were diagnosed with large-FN via NCS.

Participants were diagnosed with small-FN if at least two of the following examinations were abnormal (9, 35):

1) The sensory part (items related to pinprick, touch pressure, vibration, joint position) of NIS-LL scale $\geq$ 1 and/or selected items of the mTCNS $\geq$ 1 (namely foot pain, numbness, tingling, temperature)

2) Abnormal warm and/or cooling threshold at the foot assessed by QST ($\geq 97^{th}$ percentile compared to normative data from age and sex-matched healthy controls)

3) Reduced IENF compared to normative values (33), and 60% and less IENF in the distal probe, compared to the proximal probe (34)

Gait and balance assessment

Gait and balance were assessed during ON and OFF medication. Study participants were first evaluated in their OFF medication states in the morning, at least 12 hours after the last dose of L-dopa, and in their ON medication states, after one to three hours from taking the first dose of L-dopa during the same day. Participants were equipped with three synchronized RehaGait inertial measurement units (IMUs, Hasomed GmbH, Magdeburg, Germany), each containing a triaxial gyroscope and tri-axial accelerometer with sampling frequencies of 100Hz. The positions of the IMUs were lower back and the lateral parts of both feet. Gait was assessed with a 20-meter straight walking and a 1080° circular walking test. The latter was conducted around a 1.2-m diameter carpet in both left and right directions (36). The Timed Up and Go (TUG) test was also performed. Postural control was assessed with 30-second trials of each, side-by-side (SS) stance on the floor and on foam, with eyes opened and eyes closed, and tandem stance on the floor. The following gait parameters were extracted using validated algorithms (37-39): from the straight and circular walking data, stride time, cadence, gait variability, gait speed, stride length, heel-strike and toe-off angles; from the TUG data, duration of turns and peak angular velocity during turns; and from the static balance data, jerk in anterior-posterior (AP) and medio-lateral (ML) directions, acceleration in AP and ML directions, velocity in AP and ML directions, as well as sway area (22, 40, 41). We computed both AP and ML directions, because they were shown to represent different pathologies or compensation strategies of the body (41, 42). Explanatory material for the gait and balance parameters used in this study is provided in the Figure 1.

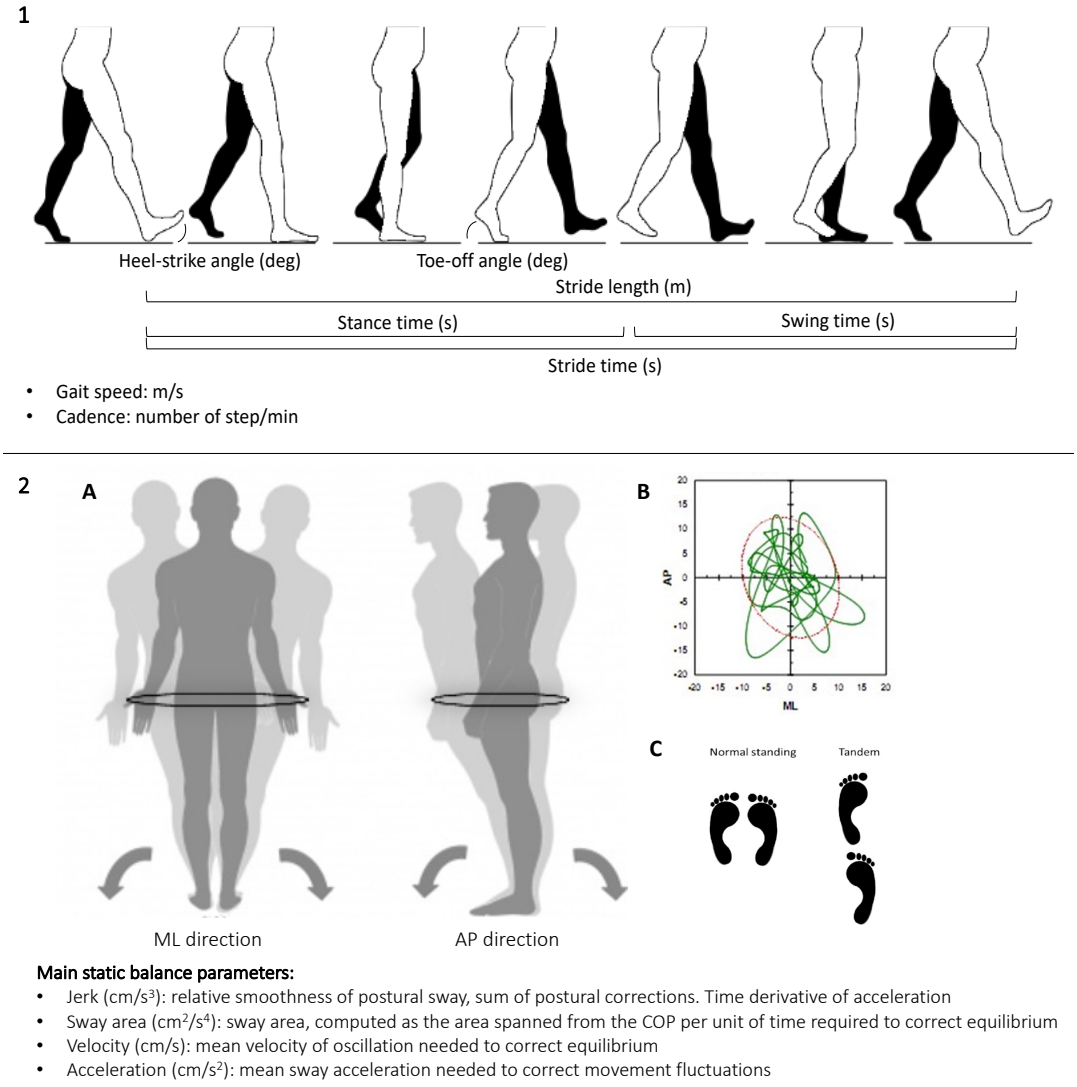


Figure 1. Visual representation of gait cycle and postural sway. (1) Gait cycle and main gait parameters extracted from IMU. **(2)** Postural sway and main balance parameters extracted from IMU **2A.** Postural sway representation in ML and AP directions and description of main balance parameters. **2B.** Representation of sway area on a balance platform. **2C.** Position of feet during static balance tasks.

2.4 Statistical analysis

Clinical and gait and balance parameters between PD-PNP and PD-noPNP were first compared using T-test or Mann-Whitney U test, as appropriate. Analysis of correlations were used to measure the linear relationship between variables and define the parameters of the final model. Normality of distribution was assessed with Shapiro-Wilk test, and variances with Levene's test.

Subsequently, a multivariate analysis of variances was used to evaluate the possible effects of PNP on gait and balance parameters. The analysis was carried out considering PD-PNP

and PD-noPNP groups as independent variables and the combined gait and balance features as dependent variables. The analysis was carried out for both OFF and ON medication states separately, and controlling for age. For the comparison of different PNP types, a univariate analysis of variance was performed using gait and balance parameters, after controlling for age. SPSS 25® software package was used. $p < 0.05$ was considered significant.

RESULTS

PD patients and prevalence of PNP

We assessed 99 consecutive PD study participants (39.4 % women) with a mean age of 67.2 (±10) years and a mean disease duration of 6.5 (±5) years (Table 1). Mean L-dopa daily dose (LEDD) was 719.1 (±10) mg.

Clinical, neurophysiological and neuropathological assessment showed that 40.4 % patients (N=40) of this PD cohort presented signs and symptoms allowing PNP diagnosis, with a predominance of small fiber neuropathy (70 % of the PD-PNP group) (Fig. 2). The main demographic and clinical characteristics of PD-PNP compared to PD-noPNP participants were not significantly different, except for UPDRS-II at ON state (p=0.004) (Table 1).

Table 1. Demographic and clinical characteristics of PD-PNP and PD-noPNP groups.

	PD-PNP (N=40)	**PD-noPNP (N=59)**	***p***
Sex n (%)	18F (45 %)	N=21F (36 %)	0.350
Age (y)	66.1 (10)	67.9 (9)	0.630
Age at PD onset (y)	59.2 (12)	61.4 (9)	0.737
Disease duration (y)	7.1 (6)	6.1 (4)	0.738
H&Y Stage	2	2	0.344
LEDD (mg)	738 (362)	706.1 (413)	0.431
UPDRS II ON	7.3 (4)	5.2 (4)	0.004**
UPDRS II OFF	8.8 (4)	7.5 (5)	0.095
UPDRS III ON	15 (9)	14.9 (8)	0.849
UPDRS III OFF	24.1 (11)	24.7 (10)	0.754
UPDRS IV	2.7 (2)	2.3 (2)	0.188
NMSS	29 (21)	33.2 (27)	0.638
DRS	122.4 (15)	123.2 (16)	0.603

Values are expressed in mean (SD). DRS: dementia rating scale; H&Y: Hoehn and Yahr stage; LEDD: Levodopa Equivalent Daily Dose; NMSS: Non-motor symptoms scale for Parkinson's disease; UPDRS: Unified Parkinson's Disease Rating Scale. Mean comparison (t-test or Mann Whitney-U test, where appropriate). **p<0.01

PNP characteristics in PD patients

Mean NIS-LL and mTCNS scores were 2.3 (±2.7) and 2.2 (±2.3), respectively. A total of 36.3 % (N=36) of the PD group showed neurological signs of PNP based on NIS-LL scale cut-off, while 12.1 % (N=12) showed noticeable neuropathic symptoms, according to mTCNS results.

Twelve percent (N=12) of PD participants showed axonal large-FN, based on NCS. No demyelinating features were observed. Sural sensory nerve mean amplitude was 14.5 (±9.3) μV, while superficial peroneal sensory nerve mean amplitude was 13.3 (±7.1) μV. Peroneal motor nerve analysis showed mean amplitude of 4.9 (±2.1) mV, mean velocity of 47.7 (±6.3) m/s and mean latency of 3.4 (±0.6) ms. QST tests revealed that 21.2 % (N=21) of study participants had impaired sensitivity to cold temperatures and heat-pain, based on temperature thresholds above the 97th percentile.

Mean IENF at the proximal thigh in the entire group was 11.5 (±3.6) and 7.3 (±3.2) at distal leg. A total of 35.3 % (N=35) PD subjects showed lower IENF at distal leg compared to normative values (33) and proximal-distal gradient above 40 %. Detailed clinical, neurophysiological and neuropathological results of the PNP investigation are provided in Supplementary Table 1.

Demographic and clinical characteristics of large and small-FN participants were not significantly different (Supplementary Table 2). Since large-FN often presents with more clinically relevant dysfunction than small-FN (5), PD participants with both small and large-FN were included in the large-FN group for further analysis.

With regard to etiology, 25 % (n=10) of the PD-PNP group presented glucose dysmetabolism, while these abnormalities were found in 20.3 % (n=12) of the PD-noPNP group (P=0.584). Glucose dysmetabolism included patients with diagnosis of Diabetes Mellitus (17.5 % of the PD-PNP cohort, n=7) and patients with HbA1c values ≥ 6.5 % (n=3)51. Three patients with Diabetes showed a multifactorial etiology of peripheral neuropathy. A total of 27.5 % of the group (n=12) (n=11) showed alterations in vitamin B6 and B12, MMA, Hcy or folic acid levels. Of this group, 63.3 % (n=7) presented vitamin B12 deficiency, 18.1 % (n=2) showed low B12 values and high B6 values, and 18.1 % (n=2) presented high vitamin B6 levels. Finally, 45 % (n=18) of the PD-PNP group had normal

blood results. In these cases with undisclosed etiology, no significant relation was found with LEDD values. One patient of PD-PNP group refused to perform the blood test (Fig 2; Supp Tab. 3).

Phospho-α-synuclein deposits were observed in 14.9 % of the study cohort (n=13), mostly in the somatosensory nerve fibers of the subepidermal plexus (n=11), but also in small nerve fibers around sweat glands (one participant), and in nerve fibers in proximity of the erector pilorum muscle (one participant). Phospho-α-synuclein deposits location were more frequent at proximal thigh level (61.5 % of participants (n= 8) compared to 15.3 % (n= 2) with an exclusive distal involvement and 23.2 % (n= 3) showing phospho-α-synuclein deposition at both proximal and distal sites (Supp. Fig. 1). Phospho-α-synuclein were present in 30.7 % of small fiber neuropathy participants (n=4). Of this group, two small fiber neuropathy subjects showed Diabetes Mellitus as main peripheral neuropathy's cause, one participant had abnormal metabolic alterations and one patient presented undisclosed peripheral neuropathy's etiology.

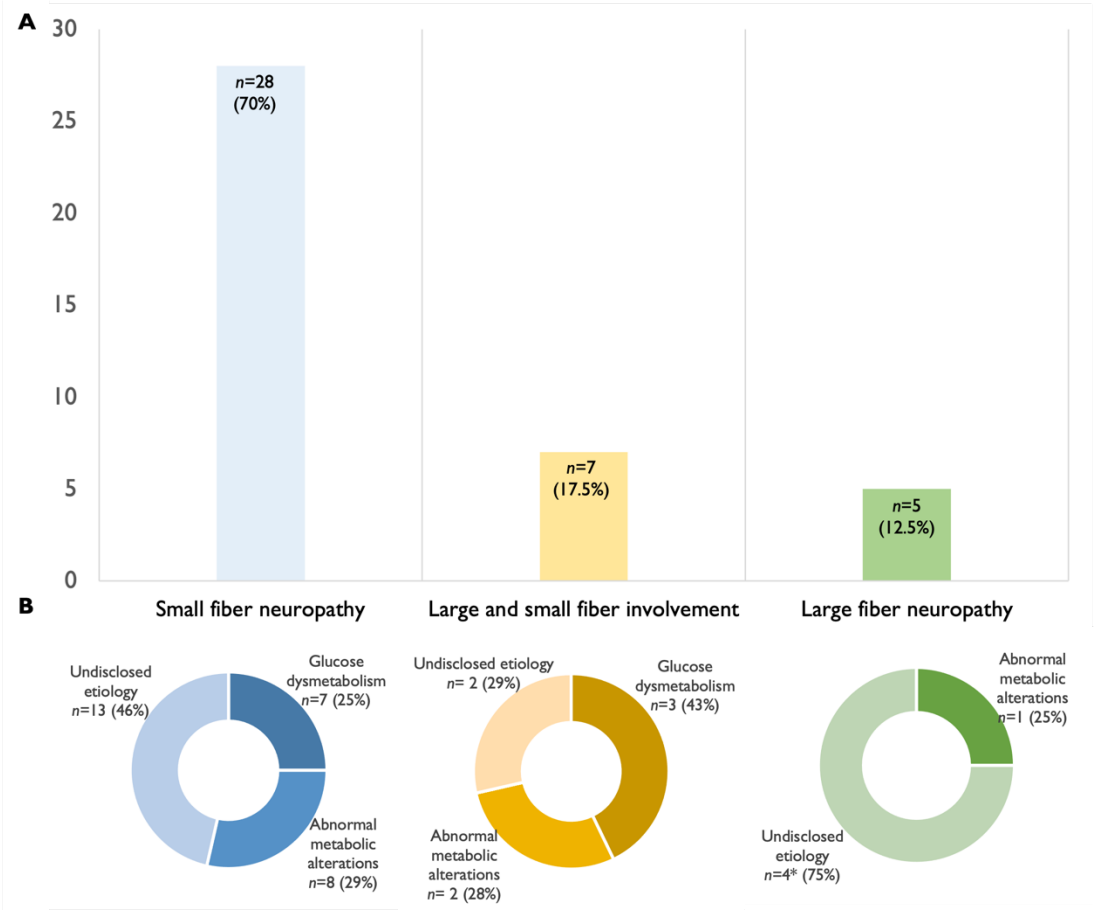


Figure 2. Prevalence of PNP in the PD cohort and related etiology. (A) Prevalence of PNP types in PD. Small fiber neuropathy (blue column) was observed in 70 % (N=28) of PD participants; Large and small fiber involvement (yellow column) represents 17.5 % (N=7) of cases; Large fiber neuropathy (green column) was observed in 12.5 % (N=5) of PD participants. **(B)** Representation of

PNP etiology for small fiber neuropathy (blue), large and fiber neuropathy involvement (yellow) and large fiber neuropathy (green) types. Values are expressed as number of subjects (%).

PNP impact on gait and balance

In order to investigate the functional impact of PNP on gait and balance, three additional PD participants with neuropathic symptoms were subsequently added to the cohort in response to a random selection. A total of 102 (43 PD-PNP and 59 PD-noPNP) participants were therefore included in the following analysis, with a mean age of 67.2 (±10) and mean disease duration of 6.6 (±5) years. The three additional PD-PNP patients did not relevantly alter the overall cohort characteristics. Gait and balance impairments were firstly analyzed at baseline during OFF medication state; subsequently, the same functional parameters were investigated at ON state, to look at medication effect.

Sensor-based gait parameters

We first performed a preliminary univariate and correlation analysis with all the gait parameters, and removed stride time, cadence and gait variability from the final multivariate model because they did not show a statistical difference between groups and for not satisfying the model's assumptions.

We observed significant differences between the PD-PNP and PD-noPNP groups on the combined dependent parameters of gait across all gait tasks (Tab. 2). In particular, at baseline, stride length (p=0.029), gait speed (p=0.005) and toe-off angles (p=0.002) were different between groups during straight walking at normal pace. During circular walking, PD-PNP participants showed slower speed (p=0.019) and smaller toe-off angles (p=0.007) at OFF state. Peak angular velocity was slower in the PD-PNP group during turns (p=0.002). At ON medication state, the above effects remained, although moderately reduced: during straight walking, all aforementioned parameters remained significantly different between groups; in circular walking and turns, toe-off angles were smaller (p=0.001) and peak angular velocity lower (p=0.01) in the PD-PNP group, compared to PD-noPNP (Supp Tab 4; Fig. 3).

Table 2. Multivariate analysis of variances of combined gait parameters controlled for age, during different gait tasks.

Gait task	Medication state	Value	F	df	Error df	Sig.
Straight walking	OFF	0.886	2.83	4	88	0.029*
	ON	0.84	3.904	4	82	0.006**

Circular walking	OFF	0.888	2.861	4	91	0.028*
	ON	0.883	2.92	4	88	0.026*
Turns	OFF	0.914	4.313	2	92	0.012*
	ON	0.944	2.765	2	93	0.027*

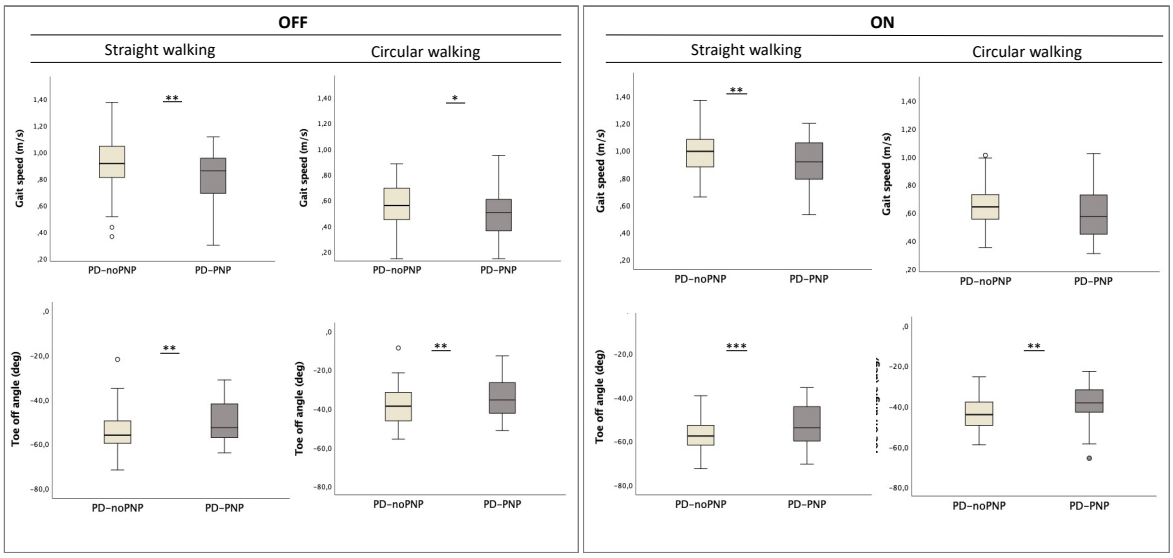


Figure 3. Gait parameters distribution in PD-noPNP versus PD-PNP. Distribution of gait speed and toe-off angles parameters during straight and circular walking tasks, at OFF (left panel) and ON (right panel) medication states, between patients with Parkinson's disease, with (PD-PNP) and without (PD-noPNP) signs of peripheral neuropathy (univariate analysis of variances). * p<0.05, **p<0.01, ***p<0.001.

Sensor-based static balance parameters

Multivariate analysis of variances on the combined dependent parameters of postural stability was performed for all the balance tasks: side by side stance, tandem stance, and stance with open and closed eyes on a foam surface. No significant differences between groups were observed during static stance on firm surface and stance with open eyes on foam. Notably, only during stance with closed eyes on foam we found a significant difference between PD-PNP and PD-noPNP on the combined dependent parameters of balance at OFF medication state, after controlling for age (Tab. 3). Specifically, jerk in both AP (p=0.028) and ML (p=0.001) directions, acceleration AP (p=0.03), velocity AP (p=0.034) and sway area (p<0.001) differed between PD-noPNP and PD-PNP. At ON medication states, no significant difference was observed between groups (Supp Tab 5; Fig 4).

Table 3. Multivariate analysis of variances of combined balance parameters controlled for age, during different balance tasks.

Balance task	Medication state	Value	F	df	Error df	Sig.
Side by side stance	OFF	0.984	0.153	7	64	0.993

	ON	0.956	0.398	7	61	0.9
Tandem stance	OFF	0.595	2.436	7	25	0.111
	ON	0.827	1.734	7	58	0.119
Open eyes stance on a foam	OFF	0.904	0.818	7	54	0.577
	ON	0.804	1.848	7	53	0.097
Closed eyes stance on a foam	OFF	0.633	4.216	7	51	0.001**
	ON	0.935	0.546	7	55	0.796

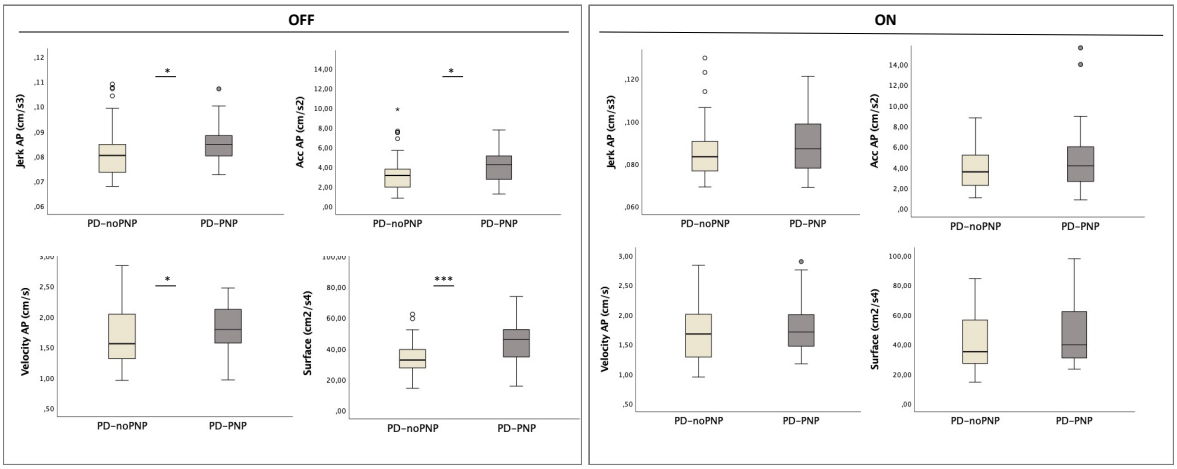


Figure 4. Balance parameters distribution in PD-noPNP versus PD-PNP. Distribution of the main balance parameters during static balance on a foam surface with closed eyes, at OFF (left panel) and ON (right panel) medication states, between patients with Parkinson's disease, with (PD-PNP) and without (PD-noPNP) signs of peripheral neuropathy (univariate analysis of variances). *p<0.05, ***p<0.001.

Comparison between small and large FN groups

An exploratory analysis was conducted to investigate differences in mobility outcomes between large and small-FN types. Both large and small-FN types contributed to impaired gait (Supp Tab 6). In particular, the large-FN group showed lower toe-off angles, compared to PD-noPNP during all gait tasks, at both OFF (p=0.001) and ON (p<0.001) medication states. In contrast, gait speed was consistently affected by both large- and small-FN, which was significantly slower compared to PD-noPNP during all gait tasks and all medication states.

With regard to postural stability, the effect of PNP was more pronounced in the large-FN group, especially in Jerk ML (p=0.004), Acc ML (p=0.005) and sway area (p<0.001) (Supp Tab 6).

DISCUSSION

In this study we found that PNP worsens gait and balance in patients with PD, regardless of its etiology or PNP type. Our study shows to the best of our knowledge, for the first time, the impact of PNP on mobility in PD using wearable health-technology.

Our comprehensive PNP assessment showed that 40.4 % of the PD population presented PNP, in line with the previously reported prevalence of 4.8 % to 55 % (5, 43). This variability may be due to differences in population size and use of different methodologies to diagnose PNP (5), such as directing only to a single type of PNP (6, 12, 14), or to clinical signs alone versus neurophysiological data (44, 45). For example, previous studies used methodologies directed only to a single type of peripheral neuropathy such as small, autonomic or large fiber neuropathy5, 17, 19. Other studies were directed to clinical signs alone versus neurophysiological data53, 54. In our cohort, we comprehensively screened for both large and small fiber neuropathy. Large fiber neuropathy was present in 12.2 % of the Parkinson's disease population. Similar proportions were also observed in previous reports: prevalence of large fiber neuropathy in idiopathic Parkinson's disease was from 6 % to 58 %7. More particularly, a recent systematic review on peripheral neuropathy in Parkinson's disease addressed an estimated prevalence of large fiber neuropathy of 16.3 % from a total of 17 studies and 1376 Parkinson's disease participants, confirming a higher incidence of peripheral neuropathy in Parkinson's disease than in the general population (7). With regard to small fiber neuropathy, we found that sensory disturbances were more frequent (35.3 % of the Parkinson's disease cohort) than large fiber neuropathy type (24, 55, 56). Small fiber neuropathy in Parkinson's disease was first investigated by different research groups (56, 57), who demonstrated reduced small fiber density in Parkinson's disease subjects. Based on IENF density, the reported prevalence of small fiber neuropathy ranged from 37% to 91% whereas the pooled estimated prevalence was 56.9 % reported in 3 studies with a total of 72 participants with Parkinson's disease (7). A decreased IENF density was also observed in 61 % of our cohort. Differently to these previous reports, in our study, we used specific and strict criteria for PNP diagnosis and classification, including NCS and a comprehensive clinical, quantitative sensory testing and neuropathological (IENF density and proximal and distal gradient) criteria to diagnose large- and small-FN, respectively (9). Our results are also comparable in terms of clinical and nerve conduction studies' profiles with results from preliminary studies (52, 53) and studies investigating only one peripheral neuropathy's type (19). We found mostly mild neuropathy signs (83.3 %) in the Parkinson's disease group, confirmed by NIS-LL cut-offs. Strongest neuropathic symptoms confirmed by the mTCNS were observed in only 12.2. % of the cohort. These results are in line with previous reports showing higher proportion of altered clinical results in Parkinson's disease

population with peripheral neuropathy compared to Parkinson's disease participants without peripheral neuropathy and healthy controls (52).

We demonstrated that the PD-PNP group did not have a more advanced age and disease duration, compared with Parkinson's patients without peripheral neuropathy. Two reasons may explain the differences between the previous studies and our results: first, peripheral neuropathy and Parkinson's disease may not be directly related, and have independent disease developments. Second, it could be that peripheral neuropathy and Parkinson's disease are related, but evolve distinctively in the central and peripheral nervous systems. Our findings were not in line with previous reports, probably because these studies excluded some peripheral neuropathy's etiologies (such as Diabetes Mellitus and inflammatory types of peripheral neuropathy), narrowing the scope of peripheral neuropathy's investigation (19), 53. The advantage of our study is that we evaluated an unbiased, consecutive series of patients, which may be more representative of the Parkinson's disease population.

Regarding etiology of peripheral neuropathy, 25% of the PD-PNP group was related to glucose dysmetabolism, which is also consistent with published literature (11, 58, 59). PD-PNP patients with a diagnosis of Diabetes Mellitus were 17.5 % (n=7) compared to 16.9 % (n=10) of the Parkinson's disease group without peripheral neuropathy (P=0.943). According to a recent surveillance data, the prevalence of Diabetes Mellitus and pre-diabetes forms among adults with more than 65 years varies from 22 % to 33 %, depending on the diagnostic criteria used (60). Our data showed a total prevalence of 22.2 % of patients with glucose dysmetabolism, which is in line with average prevalence of the elderly population. Another frequent cause of peripheral neuropathy are metabolic alterations, that were present in less than one third of the cohort. Low levels of vitamin B12 have already been reported in studies on the etiology of peripheral neuropathy in Parkinson's disease population (20,61). Levodopa toxicity has been considered a contributing factor to peripheral neuropathy in Parkinson's disease patients (22). In our cohort we did not find a significant difference in terms of mean Levodopa daily doses (LEDD) between Parkinson's disease patients with and without peripheral neuropathy (P=0.431). Moreover, methylmalonic acid (MMA) and homocysteine (Hcy) levels of the PD-PNP group with undisclosed etiology were within the normal range, and no significant correlation was found with LEDD values. Hence, a causal relationship with Levodopa was not considered in our group of patients with peripheral neuropathy (22,62). We also found a low prevalence of phospho-α-synuclein deposits in our cohort, with a higher percentage in the proximal thigh area, compared with the distal leg. Due to the low number of active phospho-α-synuclein-small fiber neuropathy subjects (n=4), and in particular of small fiber neuropathy subjects with undisclosed etiology (n=1), α-synuclein deposition was not considered directly associated with the pathophysiology of peripheral neuropathy.

Importantly, we found that PNP had a functional impact on gait during all gait tasks. During straight walking, PD-PNP patients presented slower gait speed, shorter stride length and smaller toe-off angles, compared to PD patients without PNP. This observation is in line with the reduced gait speed and increased risk of falling reported in earlier studies on PNP patients (46-49). Similar results related to gait speed and stride length were also shown by Beaulieu et al (20) in a small cohort of PD participants with PNP. This promising but still preliminary work had less strict diagnostic criteria, based on signs and symptoms and gait assessment in pressure mapping walkway of only 8 meters, which limited the assessment of different gait tasks or specific parameters such as foot angles. We used a more comprehensive PNP assessment protocol and, supported by wearable health technology, we also observed gait deficits during several other gait tasks (such as circular gait and turns) allowing for a more ecological functional assessment.

Smaller toe-off angles during gait were also reported by Hazari et al (50) in a cohort of patients with PNP: the study showed that PNP participants walked with greater knee flexion angles than healthy controls, which may be associated with musculoskeletal changes as a consequence of motor PNP, resulting in weakness and tightness of flexors muscles. Although considering that the patient population studied is different, this study also showed no significant differences in relation to heel strike angles, consistent with our results. These results suggest a more cautious gait in PD-PNP patients (smaller toe-off angle) that, in our case, may be not due to muscular weakness of the extensor muscle of the lower leg (normal heel strike angle) suggesting that, along with motor impairment, sensory and proprioceptive neuropathy may interfere and contribute to such finding.

Overall, our results confirmed that loss of somatosensory function significantly affected gait, both in more 'automatic' conditions, such as straight walking at normal pace, as well as during more demanding tasks, such as circular walking and turning. A more impaired gait in the PD-PNP group during straight walking may be related to the inability of the neuromuscular control system to respond to environmental influences when attention is reduced, such as when gait is more automatic (22). Also at ON medication state, PD-PNP gait remained significantly impaired, compared to PD-noPNP, as also evidenced clinically.

PD-PNP patients also had worse performance during static balance tasks. This was particular evident in more challenging tasks with closed eyes stance on a foam, where PD-PNP patients presented greater Jerk, acceleration, velocity and sway area values. This observation is best explained by reduced proprioception that cannot be compensated by visual feedback (22). The obvious increased reliance on vision of PD-PNP subjects to have more postural control could also reflect a sensory re-weighting problem. One of the parameters that best discriminated postural control between PD-PNP and PDnoPNP

groups was Jerk. Jerk is the sum of active postural corrections to maintain static balance, and represents a measure of smoothness of static balance (39). Studies in PNP patients suggest that increased sway in the AP direction is associated with increased movement in the hip joint ("hip strategy") (42, 51-53). We found that most parameters related to static balance, particularly Jerk, acceleration and velocity, were significantly different in the AP direction, confirming the concept that PNP subjects may predominantly show a hip strategy to compensate for the existing balance deficits (54). Significant effects of PNP on static balance were observed only at OFF medication state, and not at ON states. This finding suggests that optimizing dopaminergic therapy has a highly relevant effect on static balance in PD patients suffering from concomitant PNP. The most plausible underlying mechanism may be the dopaminergic system compensation for the lack of information from the somatosensory system, which is caused by PNP. However, a link between the effect of L-dopa on the basal ganglia system and somatosensory feedback has never been established. We argued that, the effect of PNP may be most evident when PD subjects are not in their best health status and during their best motor performance (at their OFF states) because the lack of dopaminergic compensation typically exacerbates motor impairments in PD (41). For this reason, it is particularly relevant to ensure an optimal dopaminergic treatment for this specific PD-PNP subgroup. Regarding the difference between PNP types and their effect on PD mobility, both PNP types contribute to impaired gait and balance, albeit at different levels. Large-FN seemed to affect foot angles and static balance more severely, likely due to more severe reduction of strength and proprioceptive feedback. Small-FN may lead to slower speed due to its progressive lack of peripheral sensation (15, 55).

The results of this study must be viewed in light of some limitations. We evaluated a cohort of PD participants from a single center University hospital. However, our Movement Disorders Outpatient Clinic receives PD patients at all disease stages, and the consecutive nature of the recruitment, with extremely high acceptance rate and large sample size, decreased potential selection biases. Second, the sample size, particularly of large FN patients, was small, not allowing more in-depth PNP types' comparison. Further studies with larger cohorts should be performed to understand the effect of PNP on quality of life, including its relation to falls.

Some strengths can also be highlighted: first, the accurate and well-defined diagnosis and clinical evaluation of PD patients by Movement Disorders' specialists, even though we acknowledge the possibility of misdiagnosis in the initial phases of the disease, (e.g., atypical parkinsonian syndromes). Second, we evaluated PNP using a comprehensive assessment with no restriction on PNP types, and based on well-defined and complete

diagnostic criteria. This enabled our understanding of the overall effect of PNP in PD and the establishment of some initial steps for its evaluation in clinical routine. Finally, the use of wearable health-technology may be an important new tool for assessing PNP in PD, allowing easier and faster assessments and monitoring, and the accurate quantification of different parameters may open perspectives in establishing cut-offs for PD-PNP gait and balance characterization.

In conclusion, the results of this study suggest that clinicians and researchers should evaluate and consider PNP in the assessment of PD, especially with regard to gait and balance difficulties as they increase PD patient disability. Gait and balance complications of PNP may be partially addressed by optimizing L-dopa therapy. Preventive PNP strategies and PNP-directed treatment, if effective, may decrease PD patient disability. In addition, applying PNP-oriented physical therapy, technical aids, physical exercise and tactile or vibratory feedback techniques may prevent PNP progression and decrease patient disability. This work provides consistent evidence for the implementation of PNP assessment and treatment optimization aiming at individualized PD patient care and quality of life improvement.

SUPPLEMENTARY MATERIAL

Supplementary table 1. Main clinical, neurophysiological and neuropathological characteristics of PNP assessment between groups.

	PD-PNP (N=40)	**PD-noPNP (N=59)**	***p***
NIS-LL	3.7 (3)	1.4 (2)	<0.001*
mTCNS	3.4 (2)	1.3 (2)	<0.001*
Sural nerve SNAP amplitude (μV)	12.5 (10)	16 (8)	0.011*
Sup. peroneal nerve SNAP amplitude (μV)	11.8 (8)	14.5 (6)	0.028*
Peroneal nerve CMAP amplitude (mV)	4.5 (2)	5.2 (2)	0.126
Peroneal nerve CMAP velocity (m/s)	47.2 (9)	48.2 (4)	0.792
Peroneal nerve CMAP latency (ms)	3.5 (0.8)	3.4 (0.5)	0.091
QST $\geq 97^{th}$ (N)	19	3	0.003*
IENFD proximal	11.4 (4)	11.5 (4)	0.88
IENFD distal	6.1 (3)	8.2 (3)	<0.001*

Values are expressed in mean (SD), except for QST value corresponding to subjects' number. CMAP: compound muscle action potential; IENFD: Intraepidermal nerve fiber density; mTCNS: modified Toronto Clinical Neuropathy Score; NIS-LL: Neuropathy Impairment Score for Lower Limbs; QST: Quantitative Sensory Test; SNAP: sensory nerve action potential; Sup: superficial; * ($p<0.05$) – statistically significant.

Supplementary table 2. Clinical and demographic characteristics of large and small-FN groups.

	Large-FN (N=12)	**Small-FN (N=28)**	***p***
Sex	4F (33.3 %)	14F (50 %)	0.374
Age	69.5 (12)	65.3 (10)	0.118
PD onset (years)	59 (17)	59.8 (10)	0.558
Disease duration (years)	7.9 (7)	6.9 (5)	0.845
H&Y	2.5	2	0.284
LEDD (mg)	768.5 (333)	725.2 (386)	0.642
UPDRS III ON	15.3 (10)	15.1 (9)	0.964
UPDRS III OFF	24.9 (14)	24.1 (11)	0.988
NMSS	28.4 (18)	29.2 (23)	0.799
DRS	125.7 (14)	120.4 (16)	0.343
NIS-LL	4.6 (3)	3.2 (2)	0.188
mTCNS	3.5 (3)	3.2 (2)	0.642
Sural nerve SNAP amplitude (μV)	3.4 (3)	16.4 (10)	<0.001*
Sup. peroneal nerve SNAP amplitude (μV)	4.1 (3)	15.2 (7)	<0.001*
Peroneal nerve CMAP amplitude (mV)	3.5 (3)	4.9 (2)	0.033*
Peroneal nerve CMAP velocity (m/s)	43.4 (15)	48.8 (4)	0.188
Peroneal nerve CMAP latency (ms)	3.4 (1)	3.6 (0.5)	0.916
QST $\geq 97^{th}$ (N)	5 (41.6 %)	14 (50 %)	0.889
IENFD proximal	9.7 (2)	12.2 (4)	0.079
IENFD distal	5.1 (2)	6.5 (3)	0.423

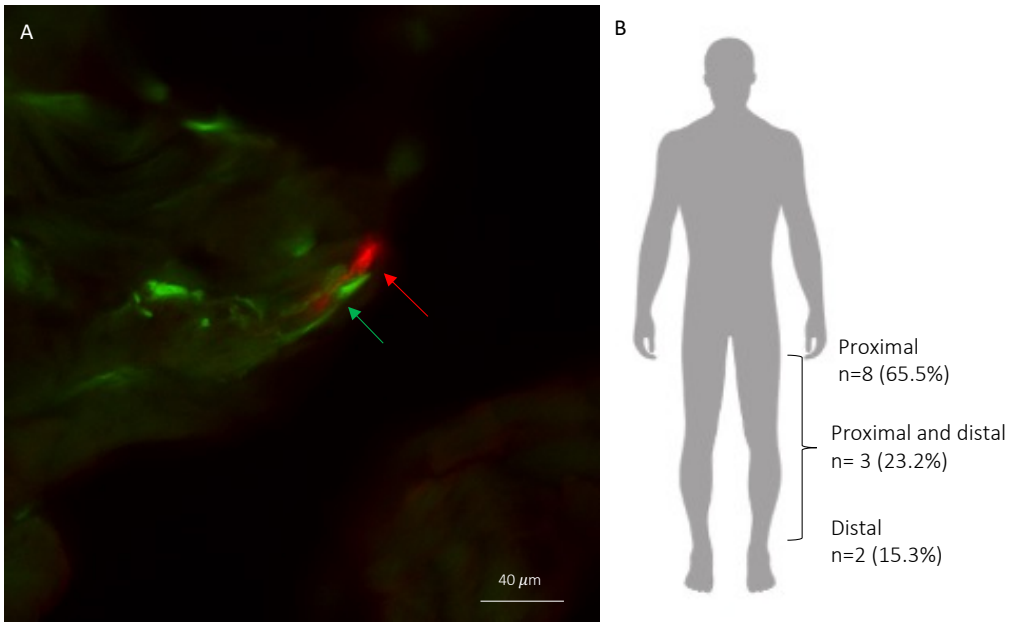
Values are expressed in mean (SD), except for QST value corresponding to subjects' number. CMAP: compound muscle action potential; DRS: dementia rating scale; H&Y: Hoehn and Yahr stage; IENFD: Intraepidermal nerve fiber density; LEDD: Levodopa Equivalent Daily Dose; mTCNS: modified Toronto Clinical Neuropathy Score; NIS-LL: Neuropathy Impairment Score for Lower Limbs; NMSS: Non-motor symptoms scale for Parkinson's disease; QST: Quantitative Sensory Test; SNAP: sensory nerve action potential; Sup: superficial; UPDRS: Unified Parkinson's Disease Rating Scale. * ($p<0.05$) – statistically significant.

Supplementary table 3. Etiological classification and laboratory work-up for PD-PNP participants.

PD-PNP patients	**PNP type**	**Etiology**	**Laboratorial findings**
#1	Small FN	Undisclosed etiology	
#2	Small FN	Abnormal metabolic alterations	VB12=182.9 pg/L
#3	Small FN	Abnormal metabolic alterations	VB12=464.9 pg/L; MMA=560 nmol/L
#4	Small FN	Abnormal metabolic alterations	VB12=232.3 pg/L; MMA=498 nmol/L
#5	Small FN	Undisclosed etiology	
#6	Small FN	Undisclosed etiology	
#7	Small FN	Undisclosed etiology	
#8	Small FN	Abnormal metabolic alterations	VB6=267 nmol/L

#9	Small FN	Undisclosed etiology	
#10	Small FN	Undisclosed etiology	
#11	Small FN	Glucose dysmetabolism	DM
#12	Small FN	Glucose dysmetabolism	HbA1c=11.2%
#13	Small FN	Undisclosed etiology	
#14	Small FN	Abnormal metabolic alterations	VB12=490 pg/L; Hcy=12.6 umol/L
#15	Small FN	Undisclosed etiology	
#16	Small FN	Undisclosed etiology	
#17	Small FN	Glucose dysmetabolism	HbA1c=7.5%
#18	Small FN	Undisclosed etiology	
#19	Small FN	Abnormal metabolic alterations	VB12=297 pg/L; MMA=468 nmol/L
#20	Small FN	Glucose dysmetabolism and abnormal metabolic alterations	DM; VB12=245 pg/L; Hcy=15.5 umol/L; VB6=160 nmol/L
#21	Small FN	Glucose dysmetabolism	DM
#22	Small FN	Abnormal metabolic alterations	VB6=205 nmol/L
#23	Small FN	Glucose dysmetabolism and abnormal metabolic alterations	DM; VB12=485 pg/L; Hcy=12.7 umol/L
#24	Small FN	Undisclosed etiology	
#25	Small FN	Undisclosed etiology	
#26	Small FN	Glucose dysmetabolism and abnormal metabolic alterations	DM; VB12=483 pg/L; MMA=1221 nmol/L; Hcy=12.3 umol/L; VB6=135 nmol/L
#27	Small FN	Abnormal metabolic alterations	VB12=329 pg/L; Hcy=15.6 umol/L
#28	Small FN	Undisclosed etiology	
#29	Both	Glucose dysmetabolism	HbA1c=7.4%
#30	Both	Undisclosed etiology	
#31	Both	Undisclosed etiology	
#32	Both	Abnormal metabolic alterations	VB12=239 pg/L; Hcy=14.7 umol/L; VB6=138 nmol/L
#33	Both	Abnormal metabolic alterations	VB12=213 pg/L; MMA=784 noml/L; Hcy=13.8 umol/L; VB6=396 noml/L
#34	Both	Glucose dysmetabolism	DM
#35	Both	Glucose dysmetabolism and abnormal metabolic alterations	DM; VB12=179 pg/L; MMA=455 nmol/L; Hcy=13.3 umol/L
#36	Large FN	Undisclosed etiology	
#37	Large FN	Abnormal metabolic alterations	VB12=271 pg/L; MMA=801 nmol/L; Hcy=11.3 umol/L
#38	Large FN	Undisclosed etiology	
#39	Large FN	Undisclosed etiology	
#40	Large FN	Undisclosed etiology**	

DM= previously diagnosed Diabetes Mellitus; **HbA1c**= Hemoglobin A1c; **Hcy**= Homocysteine; **MMA**= Methylmalonic acid; **VB6**= vitamin B6; **VB12**= vitamin B12; ** due to incomplete investigation.



Supplementary Figure1. Phospho-α-synuclein staining and localization. (A) Photomicrograph of skin biopsy of one PD participant double stained with anti-PGP9.5 (red) and anti-phospho-α-synuclein (green). Note colocalization phospho-α –synuclein (green arrows) in a nerve fiber (red arrows) of the subepidermal plexus. Images were acquired via motorized widefield fluorescence microscope at 40x/0.60 objective (Leica DMI6000, Leica Microsystems). **(B)** Phospho-α-synuclein localization (n, %).

Supplementary table 4. Estimated means and univariate analysis of variances of the main gait parameters during straight walking, circular walking and turns at OFF and ON medication states.

Straight walking

	Medication state		Mean	Std. Error	95% Confidence Interval		Univariate test
					Lower Bound	Upper Bound	
Stride length (m)	OFF	PD-noPNP	0.964	0.026	0.913	1.014	p=0.029*
		PD-PNP	0.878	0.029	0.819	0.936	
	ON	PD-noPNP	1.025	0.025	0.975	1.075	p=0.013*
		PD-PNP	0.929	0.028	0.873	0.986	
Gait speed (m/s)	OFF	PD-noPNP	0.922	0.023	0.877	0.967	p=0.005**
		PD-PNP	0.822	0.026	0.77	0.874	
	ON	PD-noPNP	0.993	0.022	0.949	1.037	p=0.006**
		PD-PNP	0.900	0.025	0.85	0.95	
Heel strike angle (deg)	OFF	PD-noPNP	11.411	0.654	10.112	12.709	p=0.826
		PD-PNP	11.192	0.751	9.701	12.682	
	ON	PD-noPNP	12.239	0.621	11.004	13.473	p=0.936
		PD-PNP	12.163	0.703	10.765	13.562	
Toe off angle (deg)	OFF	PD-noPNP	-54.865	1.099	-57.048	-52.683	p=0.002**
		PD-PNP	-49.633	1.261	-52.139	-47.127	
	ON	PD-noPNP	-57.932	0.997	-59.914	-55.949	p<0.001***
		PD-PNP	-51.914	1.129	-54.159	-49.668	

Circular walking

	Medication state		Mean	Std. Error	95% Confidence Interval		Univariate test
					Lower Bound	Upper Bound	
	OFF	PD-noPNP	0.593	0.016	0.56	0.626	p=0.28
		PD-PNP	0.566	0.019	0.529	0.603	

Stride length (m)	ON	PD-noPNP	0.679	0.02	0.638	0.719	p=0.116
		PD-PNP	0.63	0.023	0.585	0.676	
Gait speed (m/s)	OFF	PD-noPNP	0.561	0.02	0.521	0.601	p=0.019*
		PD-PNP	0.489	0.023	0.444	0.534	
	ON	PD-noPNP	0.66	0.021	0.619	0.701	p=0.057
		PD-PNP	0.6	0.023	0.553	0.646	
Heel strike angle (deg)	OFF	PD-noPNP	6.198	0.528	5.15	7.246	p=0.773
		PD-PNP	5.968	0.597	4.783	7.154	
	ON	PD-noPNP	7.295	0.45	6.401	8.188	p=0.341
		PD-PNP	6.646	0.506	5.642	7.65	
Toe off angle (deg)	OFF	PD-noPNP	-38.717	1.168	-41.036	-36.397	p=0.007**
		PD-PNP	-33.852	1.321	-36.476	-31.229	
	ON	PD-noPNP	-43.685	1.054	-45.779	-41.591	p=0.001**
		PD-PNP	-38.464	1.184	-40.816	-36.112	
Peak angular velocity (deg/s)	OFF	PD-noPNP	58.27	1.921	54.456	62.084	p=0.002**
		PD-PNP	48.997	2.153	44.722	53.271	
	ON	PD-noPNP	59,85	1.726	56.42	63.279	p=0.01*
		PD-PNP	54,397	1.922	50.579	58.215	

Turns (TUG assessment)

	Medication state		Mean	Std. Error	95% Confidence Interval		Univariate test
					Lower Bound	Upper Bound	
Duration of turns	OFF	PD-noPNP	3.161	0.118	2.926	3.396	p=0.007**
		PD-PNP	3.655	0.136	3.386	3.924	
	ON	PD-noPNP	3.049	0.098	2.854	3.244	p=0.042*
		PD-PNP	3.358	0.113	3.133	3.583	
Peak angular velocity (deg/s)	OFF	PD-noPNP	58.27	1.921	54.456	62.084	P=0.002**
		PD-PNP	48.997	2.153	44.722	53.271	
	ON	PD-noPNP	59,85	1.726	56.42	63.279	P=0.01*
		PD-PNP	54,397	1.922	50.579	58.215	

Covariates appearing in the model are evaluated at the following values: Age = 67.13.

Supplementary table 5. Estimated means and univariate analysis of variances of balance parameters during closed eyes on the foam condition at OFF medication state.

Closed eyes OFF		**Mean**	**Std. Error**	**95% Confidence Interval**		**Univariate test**
				Lower Bound	**Upper Bound**	
Jerk AP (cm/s^3)	PD-noPNP	0.081	0.002	0.078	0.084	p=0.028*
	PD-PNP	0.084	0.002	0.08	0.088	
Jerk ML (cm/s^3)	PD-noPNP	1.131	0.044	1.044	1.219	p=0.001**
	PD-PNP	1.392	0.053	1.287	1.497	
Acceleration AP (cm/s^2)	PD-noPNP	3.115	0.278	2.558	3.672	p=0.03*
	PD-PNP	3.992	0.334	3.324	4.66	
Acceleration ML (cm/s^2)	PD-noPNP	0.086	0.001	0.083	0.088	p=0.297
	PD-PNP	0.088	0.002	0.084	0.091	
Velocity AP (cm/s)	PD-noPNP	1.518	0.057	1.403	1.632	p=0.034*
	PD-PNP	1.715	0.069	1.577	1.852	
Velocity ML (cm/s)	PD-noPNP	5.935	0.574	4.786	7.084	p=0.578
	PD-PNP	4.689	0.689	3.31	6.069	

Sway area (cm^2/s^4)	PD-noPNP	31.121	1.895	27.328	34.914	p<0.001***	
	PD-PNP	43.69	2.274	39.138	48.242		

Covariates appearing in the model are evaluated at the following values: Age = 67.13.

Supplementary table 6. Comparison of gait and balance parameters between large and small FN groups.

Straight walking

Gait parameter	Medication state	PNP types		Mean Difference	Std. Error	Sig.	95% Confidence Interval for Difference	
							Lower Bound	Upper Bound
Stride length (m)	OFF	PD-noPNP	Large FN	0.112	0.062	0.074	-0.011	0.235
		PD-noPNP	Small FN	0.067	0.046	0.148	-0.024	0.159
		Large FN	Small FN	-0.044	0.067	0.513	-0.178	0.09
Stride length (m)	ON	PD-noPNP	Large FN	0.117	0.055	0.037*	0.007	0.226
		PD-noPNP	Small FN	0.079	0.04	0.052	-0.001	0.16
		Large FN	Small FN	-0.037	0.06	0.535	-0.156	0.081
Gait speed (m/s)	OFF	PD-noPNP	Large FN	0.123	0.057	0.032*	0.011	0.236
		PD-noPNP	Small FN	0.086	0.042	0.046*	0.002	0.17
		Large FN	Small FN	-0.037	0.062	0.546	-0.16	0.085
Gait speed (m/s)	ON	PD-noPNP	Large FN	0.1	0.048	0.041*	0.004	0.196
		PD-noPNP	Small FN	0.092	0.035	0.011*	0.021	0.162
		Large FN	Small FN	-0.008	0.052	0.877	-0.112	0.096
Toe-off angle (deg)	OFF	PD-noPNP	Large FN	-8.686	2.438	0.001**	-13.53	-3.843
		PD-noPNP	Small FN	-3.596	1.851	0.055	-7.274	0.081
		Large FN	Small FN	5.09	2.659	0.059	-0.193	10.373
Toe-off angle (deg)	ON	PD-noPNP	Large FN	-9.383	2.311	<0.001***	-13.978	-4.788
		PD-noPNP	Small FN	-4.657	1,652	0.006**	-7.941	-1.373
		Large FN	Small FN	4.725	2,475	0.06	-0.196	9.647

Circular walking

Stride length (m)	OFF	PD-noPNP	Large FN	0.015	0.036	0.682	-0.057	0.086
		PD-noPNP	Small FN	0.036	0.028	0.193	-0.019	0.091
		Large FN	Small FN	0.021	0.04	0.59	-0.057	0.1
Stride length (m)	ON	PD-noPNP	Large FN	0.037	0.046	0.416	-0.054	0,129
		PD-noPNP	Small FN	0.059	0.034	0.089	-0.009	0.127
		Large FN	Small FN	0.022	0.05	0.664	-0.077	0.12
Gait speed (m/s)	OFF	PD-noPNP	Large FN	0.062	0.044	0.167	-0.026	0.149
		PD-noPNP	Small FN	0.082	0.034	0.019*	0.014	0.149
		Large FN	Small FN	0.02	0.048	0.682	-0.076	0.116
Gait speed (m/s)	ON	PD-noPNP	Large FN	0.009	0.046	0.85	-0.083	0.1
		PD-noPNP	Small FN	0.084	0.035	0.017*	0.015	0.152
		Large FN	Small FN	0.075	0.05	0.135	-0.024	0.174

Toe-off angle (deg)	OFF	PD-noPNP	Large FN	-6.731	2.577	0.01*	-11.847	-1.615
		PD-noPNP	Small FN	-3.949	1.983	0.049*	-7.887	-0.011
		Large FN	Small FN	2.782	2.811	0.325	-2.8	8.364
Toe-off angle (deg)	ON	PD-noPNP	Large FN	-5.712	2.324	0.016*	-10.326	-1.099
		PD-noPNP	Small FN	-5.371	1.735	0.003**	-8.815	-1.927
		Large FN	Small FN	0.342	2.531	0.893	-4.683	5.366
Peak ang vel (deg/s)	OFF	PD-noPNP	Large FN	13.489	8.699	0.124	-3.783	30.761
		PD-noPNP	Small FN	14.545	6.688	0.032*	1265	27.824
		Large FN	Small FN	1.056	9.489	0.912	-17.785	19.897
Peak ang vel (deg/s)	ON	PD-noPNP	Large FN	16.806	9.331	0.075	-1.722	35.334
		PD-noPNP	Small FN	16.121	6.973	0.023*	2.276	29.965
		Large FN	Small FN	-0.685	10.145	0.946	-20.829	19.458

Static balance

Balance parameter	Medication state	PNP types		Mean Difference	Std. Error	Sig.	95% Confidence Interval for Difference	
							Lower Bound	Upper Bound
Jerk AP (cm/s^3)	OFF	PD-noPNP	Large FN	-0.005	0.004	0.206	-0.013	0.003
		PD-noPNP	Small FN	-0.003	0.003	0.261	-0.008	0.002
		Large FN	Small FN	0.002	0.004	0.649	-0.006	0.01
Jerl ML (cm/s^3)	OFF	PD-noPNP	Large FN	-0.331	0.112	0.004**	-0.554	-0.107
		PD-noPNP	Small FN	-0.125	0.088	0.16	-0.299	0.05
		Large FN	Small FN	0.206	0.126	0.106	-0.045	0.458
Acc AP (cm/s^2)	OFF	PD-noPNP	Large FN	-0.398	0.617	0.521	-1.628	0.832
		PD-noPNP	Small FN	-0.959	0.492	0.055	-1.939	0.022
		Large FN	Small FN	-0.561	0.696	0.423	-1.948	0.827
Acc ML (cm/s^2)	OFF	PD-noPNP	Large FN	-0.008	0.003	0.005**	-0.013	-0.002
		PD-noPNP	Small FN	0	0.002	0.885	-0.004	0.004
		Large FN	Small FN	0.007	0.003	0.013*	0.002	0.013
Vel AP (cm/s)	OFF	PD-noPNP	Large FN	-0.285	0.15	0.062	-0.585	0.014
		PD-noPNP	Small FN	-0.094	0.109	0.387	-0.311	0.122
		Large FN	Small FN	0.191	0.165	0.25	-0.137	0.519
Vel ML (cm/s)	OFF	PD-noPNP	Large FN	0.093	1.217	0.94	-2.333	2.518
		PD-noPNP	Small FN	1.21	0.906	0.185	-0.594	3.015
		Large FN	Small FN	1.118	1.334	0.405	-1.541	3.777
Sway area (cm^2/s^4)	OFF	PD-noPNP	Large FN	-17.145	4.563	<0.001***	-26.258	-8.033
		PD-noPNP	Small FN	-8.854	3.281	0.009**	-15.407	-2.302
		Large FN	Small FN	8.291	5.003	0.102	-1.7	18.282

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CHAPTERS 6.

ADVANTAGES OF AN AUTOMATED METHOD COMPARED WITH MANUAL METHODS FOR THE QUANTIFICATION OF INTRAEPIDERMAL NERVE FIBER IN SKIN BIOPSY

This Chapter was adapted from the published work:

Corrà MF, Sousa M, Reis I, Tanganelli F, Vila-Chã N, Sousa AP, Magalhães R, Sampaio P, Taipa R, Maia LF. Advantages of an Automated Method Compared With Manual Methods for the Quantification of Intraepidermal Nerve Fiber in Skin Biopsy. J Neuropathol Exp Neurol. 2021 May 26:nlab045. doi:10.1093/jnen/nlab045.
Full Open Access publication.

ABSTRACT: Intraepidermal nerve fiber density (IENFD) measurement in skin biopsy is performed manually by one to three operators. The aim of this study was to develop an automated method for fast IENFD, with low operator-dependency, to improve diagnostic accuracy and applicability in clinical practice.

Skin biopsy specimens were stained with PGP 9.5 axonal marker, and imaged using a widefield fluorescence microscope. IENFD was first determined manually by three independent observers. Subsequently, images were processed in their Z-max projection, and intradermal line was delineated automatically. IENFD was calculated automatically (Fluorescent Images Automated Counting - FIAC) and compared with manual counting on the same fluorescence images (Fluorescent Images Manual Counting - FIMC) and with the Classical Manual Counting (CMC).

A total of 60 skin biopsy specimens were analyzed. FIMC showed lower variability among observers compared to CMC (ICC=0.996 vs 0.950). FIMC and FIAC showed high reliability (ICC= 0.999). A moderate-to-high Interclass correlation (ICC=0.705) was observed between CMC and FIAC counting. The algorithm's process took on average 15 sec to perform FIAC counting, compared to 10 min for FIMC counting. We developed an automated method to rapidly and reliably detect small nerve fibers in skin biopsies with clear advantages over the classical manual technique.

Keywords: automated method; intraepidermal nerve fiber density; skin biopsy.

INTRODUCTION

A punch biopsy of the skin is a safe and minimally invasive diagnostic procedure to access small-diameter nerve fibers in the human skin, and it is recommended for the assessment of small fiber neuropathy (SFN), which affects 0.1% of the general population (1–3). Intraepidermal nerve fiber density (IENFD) is determined by measuring the number of small nerve fibers crossing the dermal-epidermal junction (or intradermal line) and calculated per millimeter. Small nerve fibers are stained with a pan-axonal antibody (PGP9.5), a marker of both myelinated and unmyelinated axons of peripheral nerves, revealing both cutaneous nerve terminals and axonal degeneration (4). This analysis has the advantage of providing a continuous quantification of nerve loss, guaranteeing the evaluation of disease progression and treatment efficacy (5). Given its high sensitivity and specificity, in the past decade IENFD has become a widely recognized technique in clinical practice and is increasingly recommended to complement physical and neurophysiological evaluation in the study of SFN patients (6). In order to standardize the use of skin biopsy in clinical practice, the European Federation of Neurological Societies (EFNS) and the Peripheral Nerve Society created a task force to define the main guidelines for this methodology: tissue processing (biopsy collection, sample preparation, and sectioning) was listed, but staining procedures and IENFD quantification methods were not standardized, generating variability (7). The quantitative determination of IENFD is performed manually by 1–3 operators (3 observers are recommended). Considering that the quantification is operator-dependent, it can result in a high interrater variability. Possible reasons for high variances among observers include: (i) the difficulty of identifying the intradermal line, which often looks blurred and not bright; (ii) the nerve fiber visualization on the maximum projection, which can generate confusion during counting; and (iii) the loss of fluorescence signal within months can affect the analysis results if repeated over time. Previous reports investigated the interrater variability of IENFD quantification. Studies focusing on variability between 2 observers found an intraclass correlation coefficient (ICC) of 0.86–0.98 (8–10), indicating a high-reliability level. Other studies have shown significant differences in IENFD counting among 3 observers and questioned the reliability of the manual counting using a more accurate statistical analysis (11). The classic technique is thus time- and human resources-consuming, limiting its use in the clinical setting. Therefore, it is necessary to investigate more standardized and less operator-dependent approaches for IENFD counting to improve reliability and standardize procedures both in research and clinical routine. With this in mind, some computerized strategies have been proposed (12), but they are generally not completely automated (4, 13), or involve private and high-cost software (14). The lack of efficient and standardized tools for IENFD counting led us to develop a custom-made approach to achieve a feasible and reliable small fiber quantification method. For this purpose, we developed a novel two-step procedure for an automated and standardized

IENFD quantification in skin biopsies: (i) a new approach for image digitization that allows a systematic identification of the intradermal line and therefore reduces variability among observers; and (ii) an algorithm for automated nerve fiber counting and IENFD measurement on fluorescence images. This approach provides a freely available and less operator-dependent procedure to be applied in research and clinical practice. The resulting work will solve the lack of manual diagnostic accuracy and apply the new method in clinical practice.

MATERIALS AND METHODS

Patients

Skin biopsies from a pool of randomly selected subjects from Centro Hospitalar Universitário do Porto (CHUP) were included in the study. All participants gave their written informed consent for the study, which was approved by the local Ethics Committee of the University Hospital of Porto (PT) in accordance with the Helsinki Declaration.

Skin biopsy and staining

Skin specimens were taken with a disposable 5 mm circular punch under sterile technique after topical anesthesia with lidocaine, and no suture was needed. The anatomical sites of skin biopsies were the lateral side of the distal leg (10 cm above the malleolus) and the proximal thigh (20 cm below the greater trochanter).

After fixation in 4% paraformaldehyde, specimens were incubated in 10% Saccharose at 4ºC overnight, then frozen with 2-Methylbutan. Immunohistochemical labeling was performed on 50-µm frozen sections using rabbit polyclonal protein-gene-product (PGP9.5) antibody (Zytomed systems, Berlin, Germany; 1:250). Indirect immunofluorescent technique with Cyanine 3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:50) as fluorescent secondary antibody was performed. The nuclei were stained with Vectashield® antifade mounting medium with DAPI. Stained sections were stored at -20°.

Biopsy fluorescence images acquisition

The same skin specimens were then imaged using a motorized widefield fluorescence microscope equipped with an HC PL FLUOTAR L 40x/0.60 objective (Leica DMI6000, Leica Microsystems). The nuclei were stained with DAPI (AT - Excitation: 340-380; BS: 400; Emission: 425 LP), and the rabbit polyclonal protein-gene-product (PGP 9.5) antibody coupled with the Cy3 was used as a pan-axonal marker (TX2 Excitation: 540-580; BS: 595; Emission: 607-683). A Z-stack was acquired with a step size of 695 nm. The stack's upper and lower limits were defined to include all fibers from the epidermis and dermis. Images

were acquired with a Hamamatsu Flash 4.2 sCMOS camera in mode binning 2x2, and the stitching was done within the LAS X Navigator extension.

Intraepidermal nerve fiber density (IENFD)

IENFD was performed and compared at three different conditions: a classical manual counting (CMC) technique on live skin biopsy' sections by three observers; manual counting by the same three observers of the fluorescence images (FIMC) acquired with a fluorescence microscope and manually counted with Fiji drawing tools; automated counting of the same fluorescence images (FIAC) by a developed algorithm. The counting methods are described in detail below:

1. Classical Manual Counting (CMC)

IENFD was determined manually for each specimen by counting directly through the oculars and focusing through the optical planes by 3 independent observers trained following published counting guidelines. Only single IENFD crossing the intradermal (dermal-epidermal) junction was counted (7). All the tissue sections were analyzed using Nikon Eclipse E400 fluorescence microscope at 40 high magnification. No image acquisition was performed. The length of the section was measured using the 2.5 objective and LAS V4.3 software. Fibers density was calculated as the number of IENFD per length of the section (IENFD/mm).

2. Fluorescence Images Manual Counting (FIMC)

A manual intradermal line (MIL) was drawn by one of the observers using Fiji drawing tools by following the epidermal cells stained in the DAPI channel. Fibers intersecting the MIL were manually counted by the same observers of the classical technique. A counting quality control was made by annotating the counted fibers with the Fiji point tool and the images were saved for further validation (15).

3. Fluorescence Images Automated Counting (FIAC)

Each stack of images was used to measure the PGP9.5 fluorescent fibers within the whole skin section and its Z maximum (Z-max) projection. Two open-source Fiji macro scripts were written to detect the intradermal line and perform automated IENFD quantification. Although the analysis was fully automatic, the scripts were written to have full user interaction, control, and validation of the main steps. The algorithm is schematized in Figure 1 and consisted of the following steps:

a) **Input images**

After microscope acquisition, images are prepared and pre-processed for further analysis.

I. Each image stack is **merged** to obtain the full skin section into a 4D mosaic image (XYZC).
II. A **scaling** factor of 0.5 is applied to all images since the original image has a non-workable size (approximately 20Gb each).
III. A **maximum Z-projection** of focus planes is applied to reduce z stacks into a 3D mosaic image (XYC)
IV. **8-bit conversion** and **scale removal** are done to apply always the same range of values in the following steps
V. Images should be oriented with the epidermal site in a right-down direction, so the user is prompted to correct image **rotation**, if necessary.
VI. Images are **split** into two different channels: the DAPI channel is used for the Intradermal line detection and the PGP channel to count the IENFD.

b) Automated intradermal line (AIL) detection

DAPI channel automatically delineates the intradermal line, following the cell nuclei reference stained in the epidermal site.

I. Correct DAPI channel illumination and apply a Gaussian blur **filter** of sigma 20 px to enhance the epidermal site.
II. In some particular cases, the full section was cropped in continuous ROI's, to overcome intensity variabilities or mounting issues. Each ROI was analyzed independently, and the result was summed up at the end.
III. The epidermal site **segmentation** is done by applying a default threshold. The user is prompt to validate and, if necessary, manually adjust for better results. The final region of interest (ROI) is segmented if the area is larger than 5000 px, with no holes and without touching the image's edges.
IV. Some ROI manipulations are done to select the intradermal line automatically (cut the ROI extremities with a shrunk bounding-box (Enlarge -10px); select the upper line; area to line; interpolate). The user is prompted to validate the final intradermal line.
V. The intradermal line (AIL) and the bounding-box are saved as a roiSet with the same name as the image file.

c) Automated IENFD counting

The number of fibers crossing the intradermal line is quantified as the PGP channel's fluorescence signal's local maxima peaks

I. Remove the PGP channel's **background noise** with the subtract background function (rolling ball radius of 100px). Apply the bounding-box saved in the previous step to get the correct intradermal line position.

II. Get the (x,y) coordinates of the AIL and measure the **perimeter** by multiplying with pixel size to get the correct intradermal line length (in millimeters)

III. Plot **AIL profile** and find the local maxima above the threshold value that is, by default, the profile's standard deviation. The user is prompted to change the threshold value, if necessary.

IV. The **(x,y) coordinates** of each fiber crossing the AIL are counted and drawn in the image for further validation.

V. A **validation** step with user visualization of the result can be done, allowing the repetition of steps c) and d) until satisfied.

VI. Image with AIL drawn and fiber's crossing and the Log file with numeric results are saved.

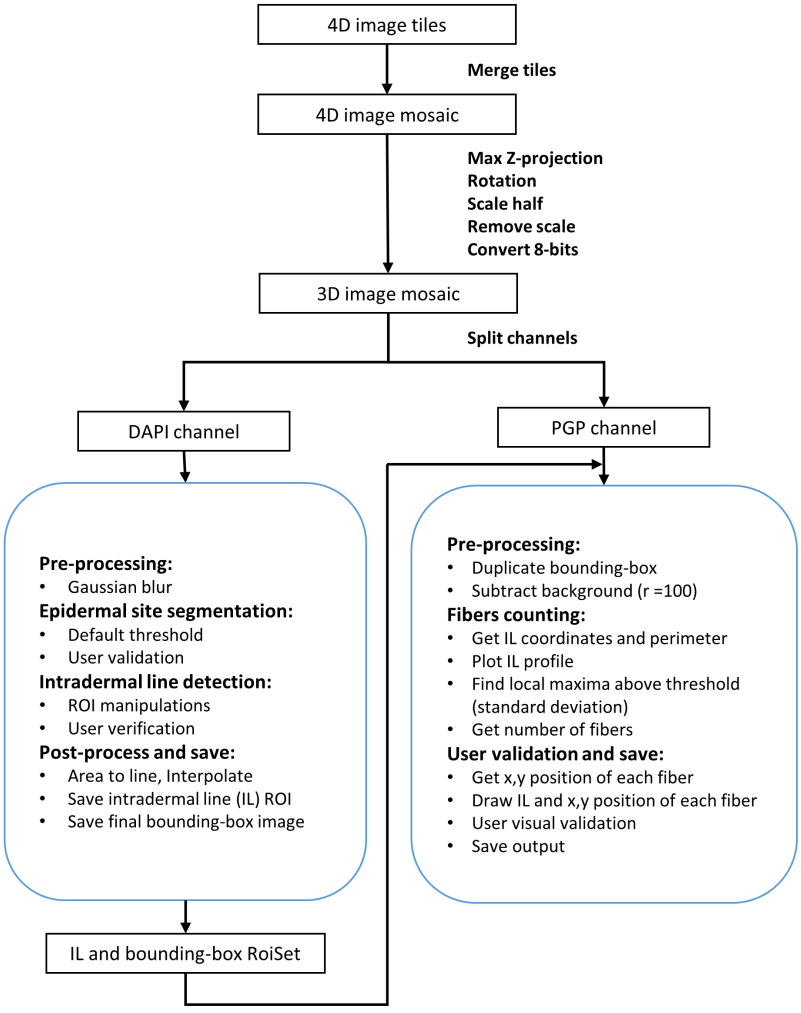


FIGURE 1. Automatic IENFD counting workflow

Validation of IENFD

IENFD is calculated as the number of IENFD per section's length (IENFD/mm). IENFD was validated in three propositions: (1) validation of IENFD Fluorescence Images Manual Counting (FIMC) with the Classical Manual Counting (CMC). (2) validation of Fluorescence Images Automatic Counting (FIAC) with the Fluorescence Images Manual Counting (FIMC). (3) validation of the automatic intradermal line (AIL) detection with the manual intradermal line (MIL) drawing. In the first proposition, the sections' length used for normalization was obtained in the classical technique. In the second proposition, the section's length was obtained from the intradermal line perimeter drawn manually. The automatic intradermal line length was compared with the manual drawn intradermal line length in the last proposition.

Statistical Analysis

The ICC (2-way mixed average measures [consistency]) and the relative intertrial variability were calculated to determine the interrater variability among the 3 observers during CMC and FIMC. Relative intertrial variability was expressed as the percentage obtained from dividing the difference between the 2 values by the mean value. Relative intertrial variability values of <10% indicate a high degree of reproducibility (10). The accuracy between each pair of observers was estimated by performing a correlation analysis (Pearson or Spearman, based on sample distributions) and the coefficients of variation (or relative standard deviation). To compare the manual counting method with the automated counting algorithm, ICC, correlation analysis (r), paired comparison and coefficients of variation were calculated. Bland-Altman plots were used to evaluate the agreement between the 2 techniques (16). All statistical analysis was performed using the SPSS 25 software package, and results were expressed in mean ± SD. p values < 0.05 were considered statistically significant.

RESULTS

A total of 60 skin biopsy specimens from a total of 10 participants with no known diagnosis of SFN were analyzed. The mean age of the subjects was 69.9 years old (20% F). IENFD on live sections with CMC technique was 8.3 (3.4).

Image processing and automated IENFD

The following section describes the automated IENFD and intradermal line detection result in the fluorescence skin biopsy images. Figure 2 illustrates a full biopsy section with PGP fluorescence highlighting the nerve fibers and DAPI fluorescence staining the epidermal site cell nucleus. Tissue thickness allowed the acquisition of images with around 30 z-focused-planes. With maximum z-projection of the middle 30 z-planes, each fiber's signal is kept and, in most of the cases, enhanced. Figures 1C and D illustrate each channel information separately.

A high portion of the acquired images presented a good signal-to-noise ratio. Images also presented a high variability of the intensity values, not only between samples but also within the same sample, as illustrated in figure 3. Different intensity histograms compromise the automatic segmentation process necessary in the detection of the epidermal site. Therefore, some images were cropped in continuous regions and analyzed separately to have less intensity variability in each region. Mounting the tissue on the coverslip could generate samples in which the biopsies' tips were slightly raised concerning the rest of the tissue. In those cases, the images were cropped to obtain most of the tissue of interest and remove the tissue tips from the analysis.

The automatic intradermal line detection was achieved in all the images, and each length was qualitatively compared with the manual intradermal line.

Figure 4 illustrates the comparison of both manual and automatic intradermal lines. Manual intradermal line (MIL) drawings was more rectilinear, while the automatic intradermal line (AIL) detection followed the epidermal site scrupulously on top of the epidermal site. As a result, the total AIL length was significantly bigger than MIL length ($p<0.001$) (Supplementary Figure 1), and the number of fibers intersecting both intradermal lines was consequently different. These differences were therefore normalized applying a correction factor of 1.2 ($\pm$0.2), which allowed the use of both MIL and AIL in the IENFD counting. Nevertheless, in order to compare the automated and the manual counting as accurately as possible, MIL was used as a fixed variable for both counting methods in the following comparisons.

The total algorithm’s process took, on average, 15 sec (depending on the image size, but not on the number of fibers) to perform the FIAC counting.

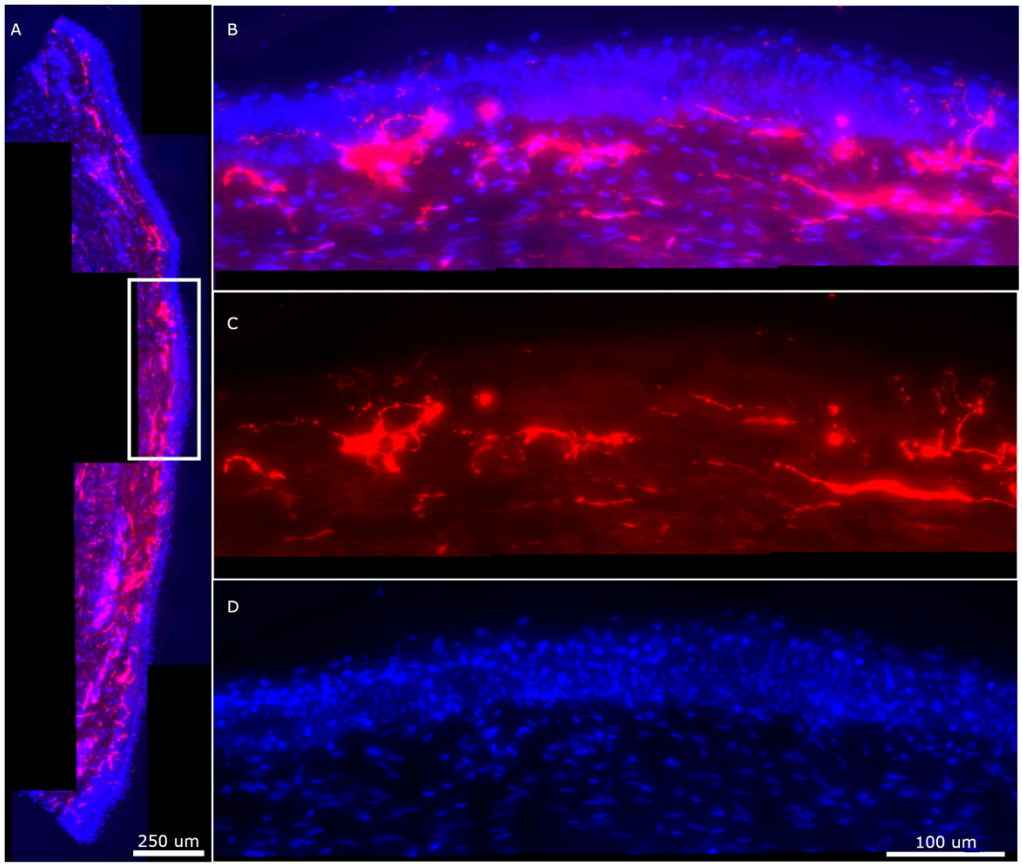


Figure 2. Original biopsy section obtained in a widefield fluorescence microscope, 40 /0.60 objective. **(A)** A middle z-plane of the 4-dimensional section. (B, C) Rotation of the white square in panel A. **(B)** Maximum Z projection; **(C)** maximum Z-projection of the PGP channel; **(D)** maximum Z-projection of the DAPI channel.

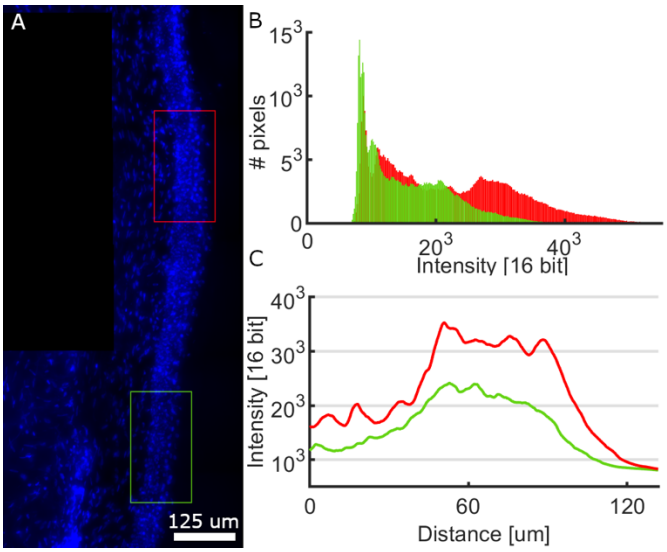


Figure 3. Intra-variability of the staining fluorescence. **(A)** A cropped section from the DAPI channel of a biopsy section; the red square delimitates a region with high-intensity values compared with the green region with low-intensity values. **(B)** Histograms of intensity value distribution from each region. **(C)** Plot profiles from each region.

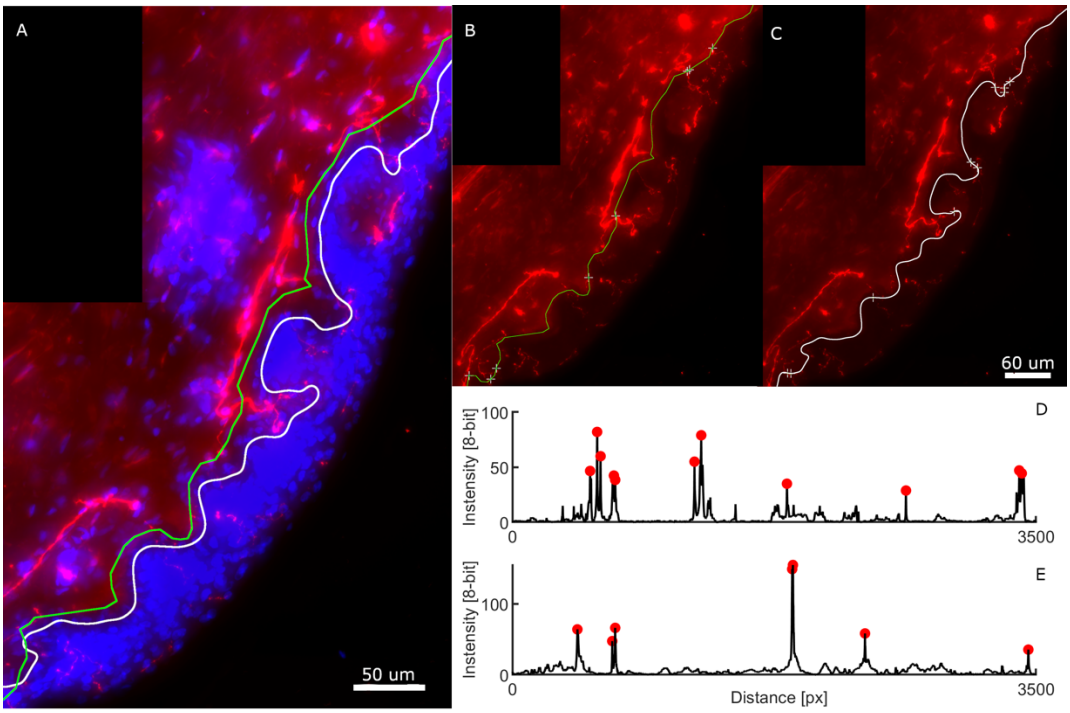


Figure 4. Automatic intradermal line detection and IENFD quantification. **(A)** Cropped section from a biopsy image of the automatic (AIL) and manual (MIL) intradermal lines (AIL in white and MIL in green). **(B, C)** Panels illustrate the detected IENFD spots crossing AIL and MIL, respectively. **(D, E)** Intradermal line profiles of AIL and MIL, respectively, with crossing fibers detected as the local maximum values (threshold = 20 [in an 8-bits range], minimum distance = 10 px).

Interrater variability between observers during manual counting

We compared the interrater variability between observers either in the Classical Manual Counting (CMC) or Fluorescent Images Manual Counting (FIMC).

a. Classical Manual Counting (CMC)

The main descriptive characteristics of the three observers' counting are described in Supplementary Table 1. Interclass correlation (ICC) among the observers was 0.950 (Table 1). The coefficient of variation among the observers was 14.7%. In terms of operator time, manual counting took on average 10 min per section for each operator.

b. Fluorescence Images Manual Counting (FIMC)

The same 60 skin biopsy specimens were acquired with a multispectral camera-based fluorescence Leica microscope, as described. After the image pre-processing and manually drawing the intradermal line, the IENFD was manually counted by the same three observers (Supplementary Table 1). Interclass correlation (ICC) among the observers was 0.996

(Table 1). The coefficient of variation among observers was 8.1%. The FIMC showed significantly lower variability among observers compared to the CMC method.
Fluorescent Images Manual Counting (FIMC) were comparable with the CMC counting in terms of time: it took on average 10 min to draw the line and manually count the fibers.

Table 1. Interrater variability among three observers for both techniques.

	CMC		**FIMC**	
	ICC=0.950		**ICC=0.996**	
	r	**RIV (SD)**	**r**	**RIV (SD)**
Observer 1 and 2	0.808***	23.4(16)%	0.966***	8.8(8)%
Observer 1 and 3	0.875***	19.4(14)%	0.949***	11.2(9)%
Observer 2 and 3	0.942***	13.7(12)%	0.948***	10(10)%

Degrees of correlation (r) and relative intertrial variability (RIV) of manual counting among three using the classical technique and fluorescence images. *<0.05; **<0.01; ***<0.001.

Comparison of the methods

a. Fluorescent Images Automated Counting versus Fluorescent Images Manual Counting (FIAC and FIMC) methods

After demonstrating that manual counting on fluorescence images showed high reliability and decreased variability among observers, Fluorescent Images Manual Counting (FIMC) and Fluorescent Images Automated Counting (FIAC) methods were compared to validate the automated algorithm (Table 2). Interclass correlation (ICC) between the two counting methods was 0.999. Correlation analysis showed a significant and robust correlation between the two methods ($r=0.995$; $p<0.001$). The mean coefficient of variation was 2.1%; no significant differences were shown between counting mean values ($p=0.817$). Bland-Altman plots (Fig. 5a) represented a strong degree of agreement between manual and automated counting, confirmed by a mean difference of -0.008 units between the two counting methods (16).

b. Fluorescent Images Automated Counting versus Classical Manual Counting (FIAC and CMC) methods

Results from the FIAC method were finally compared with the CMC method, with the aim to apply the new method in clinical practice (Tab 2). A moderate-to-high Interclass correlation between the two methods was observed (ICC=0.705) and a significant degree of correlation ($r=0.651$; $p<0.001$). The coefficient of variation was 31.6%. Fig. 5b) showed a moderate degree of agreement between the counting techniques, observed in a mean difference of -5.1 units.

Lastly, single IENFD values from both manual (CMC) and FIAC counting methods were compared to evaluate the new counting method's application in clinical practice. To define a normalization formula, we compared the FIAC results with the mean observer CMC results. Normalization is applied such that the automated detection rate becomes an indicator of IENFD, applicable in clinical routine. An initial regression analysis considered all the 60 biopsies, which coefficient was adjusted based on its variability, for all the skin biopsies' counting. Fig. 6 shows the final regression line (blue line) for all the biopsies with a slope of 0.83 and y-intercept of b=1.52. A standard error of 0.2 confirmed the feasibility of the normalization for this sample size (Fig 6).

Table 2. IENFD using three distinctive counting methods.

	CMC	**FIMC**	**FIAC**
Mean (SD)	8.3 (3.4)	13.5 (7.4)	13.5 (7.2)
95% Confidence Interval	[7.4-9.2]	[11.5-15.4]	[11.6-15.3]
Median	7.7	9.7	10
IQR	[5.9-10.8]	[8-20.9]	[8.2-20.6]

Legend: CMC: Classical technique manual counting; FIMC: Fluorescence images manual counting; FIAC: Fluorescence images automated counting; IQR: interquartile range.

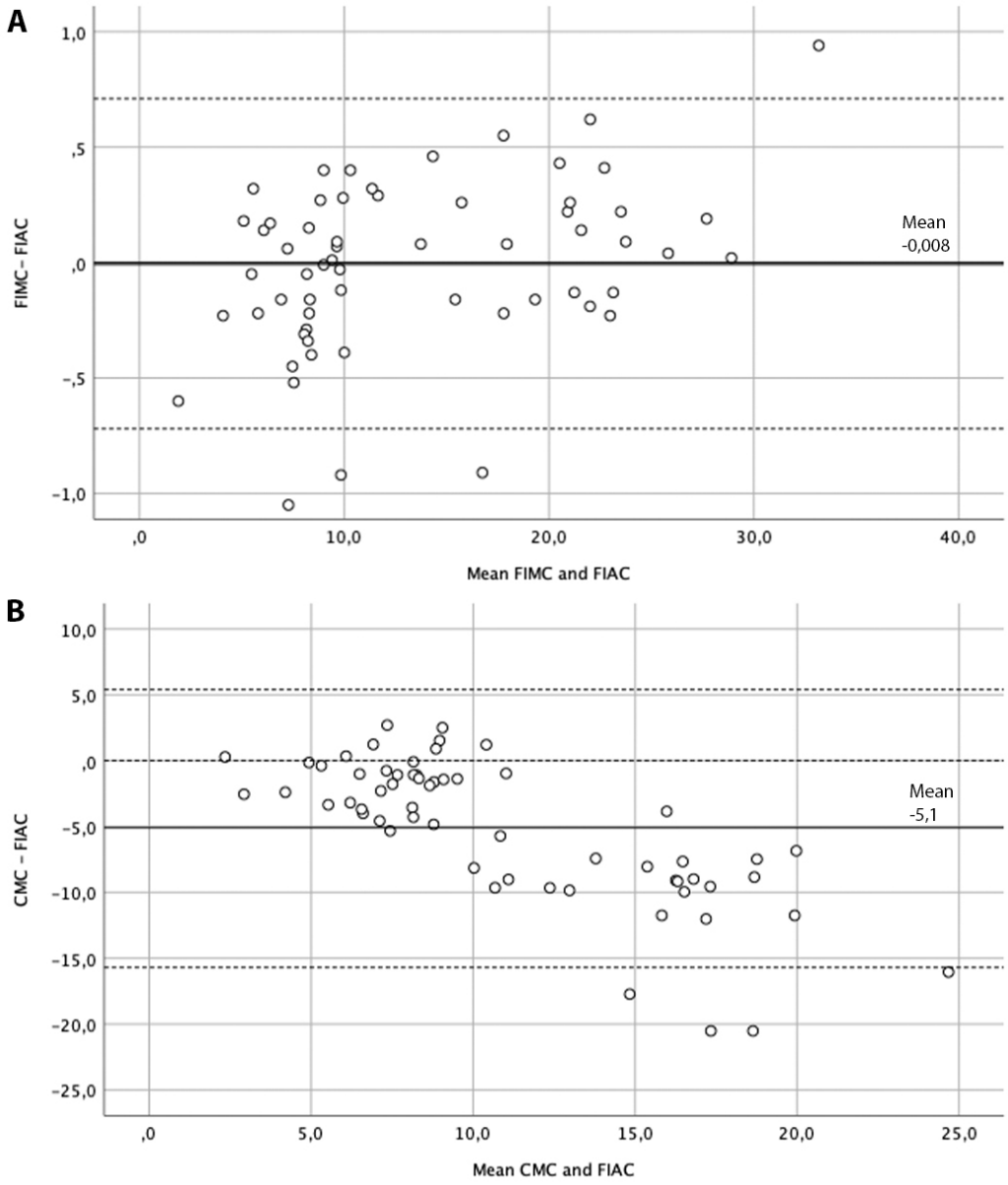


Figure 5. Agreement analysis between the counting methods. The X-axis represents the difference between the 2 methods and Y-axis is the mean of the 2 methods. **(A)** Agreement analysis between FIMC and FIAC. The bias of –0.008 units (bold line) is represented by the gap between the X-axis, corresponding to zero differences, and the parallel line to the X-axis at –0.008 units. **(B)** Agreement analysis between CMC and FIAC. The bias is –5.47 units. The limits of agreement are represented in dotted lines in both figures.

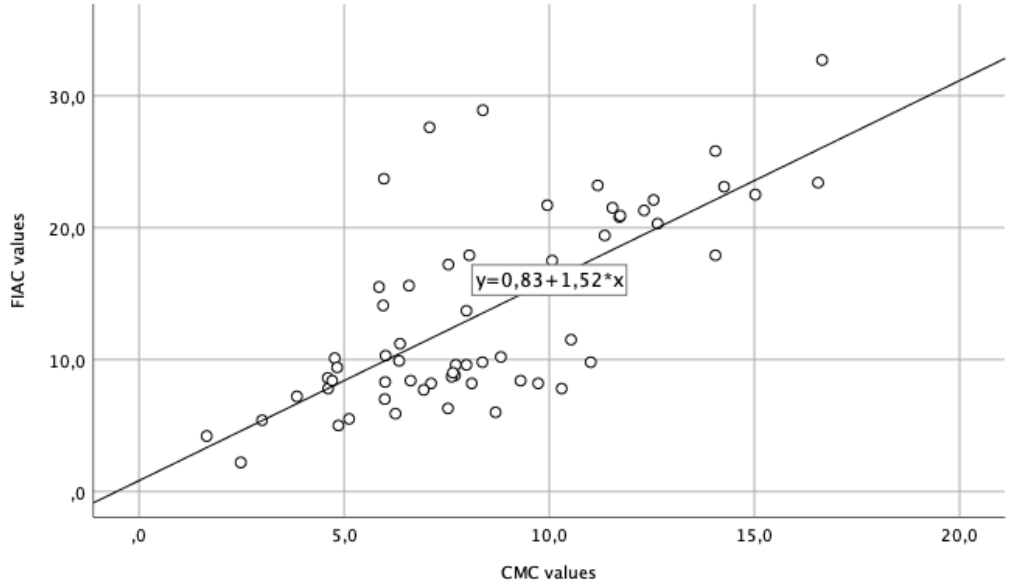


Figure 6. Regression analysis between manual and automated counting. The black line represents the best-fit regression. A normalization of 0.83 was found through the linear regression slope.

DISCUSSION

IENFD quantification in skin constitutes an excellent method to investigate SFN (7, 17, 18). This methodology consists of specialized manual procedures of staining and counting, which require laborious methodological skills and training, and therefore prone to human error when applied in conventional laboratories (19). Here, we describe for the first time an automated method for IENFD in skin biopsies using fluorescence images acquired in widefield microscopes. This method automatically obtains the nerve fiber density estimation quickly and reliably from PGP's axon fluorescence in the epidermis and dermis instead of observer-dependent visualization (20). We believe that this method has a high potential for clinical application. One of the major advantages of the method is the use of free and open-source software; the ImageJ/Fiji program, a widely used software for microscope fluorescent image analysis (21). Fiji is supported by many online tutorials, allowing low complexity, reliability, and reproducibility with potential applicability for further quantification analysis (13). Other laboratories can use the developed algorithms to standardize the IENFD methodology or it can be easily adapted and upgraded depending on the requirements. In addition, any type of brightfield microscope and related software can be used to perform the acquisition and quantification, which confirms this method's general applicability in research and clinical facilities. The digital long-term storing of patient data and information has recently become available in hospitals and pathology laboratories. The proposed automated method saves and stores this information to patients' folders and shares it with other operators, favoring second opinions, and reliability. Moreover, this new

approach provides an easy workflow for clinicians and researchers. It enables quantifying IENFD of a skin section in about 15 seconds (depending on the image size, not on the number of fibers nor biopsy site), allowing a faster quantification compared with manual counting (about 10 minutes), and improving what was proposed by Seger et al (14), where the average time needed for IENFD of one section was about 3 minutes. Overall, the user-friendly characteristics (e.g. the easy workflow and the quick repeatability), the reduction in processing time, and the significant degree of correlation with manual counting results (for comparisons over time) are the main advantages of the proposed method. Images can also be easily stored without losing intensity over time compared with the operator-dependent manual classification technique.

Methodological Considerations

There are several methodological considerations to highlight. First, we performed indirect immunofluorescence staining of small nerve fibers with PGP9.5 antibody instead of other techniques, such as immunohistochemistry visualization. The main reason for this choice is that immunofluorescence makes easier identification of the exact point where fibers cross the intradermal junction, allowing more accurate IENFD counting than immunohistochemistry. Additionally, this technique allows 3D analysis via fluorescence microscopy with higher resolution, making it suitable for detecting smaller variations (18). Fluorescent staining techniques are therefore the preferred choice for the development of new automated counting methods, allowing better accuracy and sensitivity. In addition, in the last decades immunofluorescence has been widely used in clinical routine. On the other hand, fluorescence vanishes over time, and for this reason previous studies focused on more stable and conventional immunoperoxidase staining for IENFD (4, 7). Although both techniques allow a useful IENFD, we opted for immunofluorescence since small nerve fiber staining after formalin fixation might be discontinuous, inducing less accurate results. DAPI staining of cell nuclei was considered as a reference for delineating the intradermal line. This choice was innovative and driven because the intradermal junction is often blurred due to thick skin biopsy sections of 50 μm, resulting in out-of-focal-plane fluorescence signals. To find a proper way to detect the intradermal junction automatically, we opted to use an anatomical reference that was easy to stain and detect. This choice was corroborated by previous reports considering epidermal cell nuclei as the dermal-epidermal reference (19, 22). We proposed a validated (compared with MIL detection) and AIL in order to efficiently and objectively quantify IENFD quickly and reliably, improving the manual drawing approach proposed in previous works (13).

Second, 2 technical aspects regarding preprocessing images need to be highlighted. We applied a maximum Z-projection in order to collect information from all stacks. This choice

was justified by the necessity to gather multiple images taken at different focal distances to ensure a greater field of depth. Previous studies adopted different techniques: Tamura et al (23) set a fixed number of 32 “layers-images” for each biopsy section, while Seger et al (14) acquired 21 z-planes separated by 2 mm. We believe that this methodology allows better detection of nerve fibers in the z-plane and increases image details as Casanova-Molla reported (13). These methodological aspects allowed us to reduce the variability between observers during FIMC (ICC = 0.996; Table 1), compared with CMC. Another technical observation is the possibility to manually select the brightness threshold during FIAC, which is also carried out in similar studies (13, 24). The most common automatization problem is distinguishing artifactual features from actual nerve fibers (25). Therefore, for a meaningful quantification of the fluorescent structures, the operator can select an appropriate threshold to avoid false positives or artifacts and ensure accuracy through operator validation. Manual threshold adjustments provided consistent measures in our sample, corresponding to very high reliability and low variability, as observed in the strong ICC and correlation values between FIMC and FIAC (ICC = 0.999 and not a significant difference mean values), also visually confirmed by the Bland-Altman plot (Fig. 5A). Concerning the clinical application, a fairly significant correlation was observed between the automated method and the manual counting with live visualization (r.0.651; $p<0.001$). This is in line with results from other studies (13, 22), which also showed a similar correlation when comparing their methodologies with manual counting. However, despite the significant correlation, our technique showed a significant difference in mean values with the classic counting, more pronounced in samples with less fibers (Fig. 5B). This can be justified by the different nature of the 2 counting methods: one technique is based on the live visualization of small nerve fibers on biopsy sections, while the automated method consists of acquiring and preprocessing images; the specific characteristics, such as z-projections and fixed intradermal line definition can increase the number of small nerve fiber detection. In light of these differences, a correction factor of 0.8 was calculated to apply the new method in clinical practice. Even though the correction factor showed small variability (SD = 0.2), it can be considered a promising preliminary step to be proved and adjusted in further analyses and with increased sample size. Some limitations need to be addressed. Fluorescence immunostaining with PGP9.5 and DAPI for cell nuclei may have a disadvantage with respect to image quality, which could lead to lack of reproducibility. Two main factors may contribute: first, immunofluorescence vanishes over time. We recommend performing staining and image acquisition in <6months to ensure optimal conditions for the efficacy of the automated method. Second, we used a free-floating staining approach, which may lead to discontinuity, especially for large biopsy sections. This approach was preferred over mounting sections directly on glass slides because it allows a better antibody

penetration and thus should be the method of choice when thicker sections are used (such as 50 mm thickness of our samples) (26). To solve quality discontinuity in some images, we analyzed them into parts and subsequently summed up the counting results. Furthermore, the costs of motorized microscopes with automated acquisition set-ups are higher than traditional fluorescence microscopes. Still, costs are variable and depend on the type of set-up chosen (type of camera, number of filters, number of objectives). However, increased costs are counterbalanced by several advantages, including better accuracy, sensitivity and reproducibility of images. Automated multispectral camera-based microscopes give brighter and more defined images and allow more easily developed new counting methods and new techniques that could also be useful in clinical routine. Overall, the higher initial investment can provide faster acquisition time, reduction of human error and the possibility for the operator to perform other different tasks at the same time. Future work will be needed to focus on the reproducibility of counting within and between different neuropathological institutions and on implementing the automated counting algorithm with deep learning techniques.

In conclusion, we have developed a method to rapidly and reliably detect small nerve fibers in skin biopsies that can be applied in biomedical research as well as clinical settings. We demonstrated that this technique first acquires well-defined fluorescence images and automatically detects the intradermal line, reducing variability among observers during manual counting. We also developed a new algorithm for automated detecting fibers, easy and quick to use, which showed strong reliability and feasibility compared with manual counting. Additionally, a preliminary normalization of values demonstrated possible applicability and comparability of the method with the classical manual technique, allowing its application in clinical settings and diagnosis. We suggest this method as a complementary approach to classical determination of IENFD raising the efficacy for a more complete and standardized diagnostic tool for SFN.

SUPPLEMENTARY MATERIAL

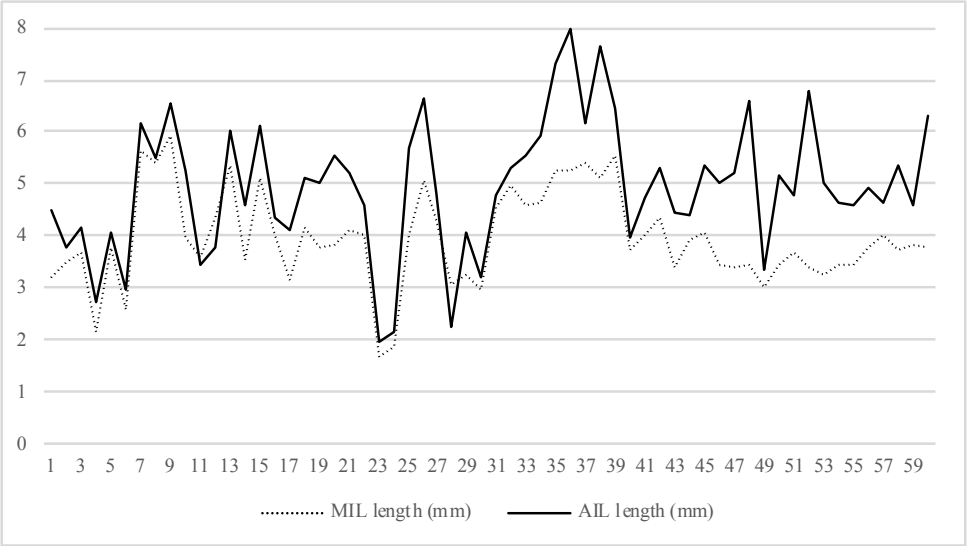
Supplementary Table 1. Descriptive characteristics of IENFD manual counting among the three observers using the classic technique and the fluorescence images.

	Observer 1	Observer 2	Observer 3	Total
Classical technique (CTMT)				
Mean (SD)	7.7 (2.9)	8.7 (3.8)	8.6 (3.8)	8.3 (3.4)
95% Confidence Interval	[6.9-8.4]	[7.7-9.7]	[7.6-9.6]	[7.4-9.2]
Median	6.8	7.8	8	7.7
IQR	[5.6-9.9]	[6-11]	[5.8-10.7]	[5.9-10.8]

Fluorescence images (FIMC)				
Mean (SD)	13.7 (7.6)	13.7 (7.4)	13 (7.3)	13.5 (7.4)
95% Confidence Interval	[11.7-15.7]	[11.8-15.6]	[11.1-14.9]	[11.5-15.4]
Median	10.7	10.4	9.5	9.7
IQR	[8-20.8]	[7.6-20.6]	[7-18.9]	[8-20.9]

IQR: interquartile range

Supplementary Figure 1.



Supplementary Figure 1. Manual intradermal line (MIL) and automated intradermal line (AIL) length values for all skin biopsy cases (N=60). Lengths are expressed in mm. AIL lengths were significantly greater than MIL values ($p<0.001$).

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CHAPTER 7.
DISCUSSION

Available treatments for PD provide symptomatic relief and attempt to improve the patient's activities of daily living during the course of the disease. Research has increasingly focused on more effective technologies to quantify PD symptoms with a new level of granularity, and in particular wearable health-technology improved monitoring of motor function in PD with objective measures.

The first aim of the project was to investigate the degree of association between supervised and unsupervised assessments during different medication states in PD. It was achieved with the elaboration of two complementary works, as a result of a collaboration with the research team of École Polytechnique Fédérale de Lausanne, Switzerland (EPFL) and Gait Up S.A. (Lausanne, Switzerland), a company specialized in the production of sensors and algorithms.

In these studies, a total of 27 PD patients were assessed in their OFF and ON medication states, and gait was evaluated in two different settings: the laboratory assessment included several gait tasks (i.e., straight walking at normal and fast pace, circular walking), and one full-day of unsupervised assessment at home. Gait speed was extracted from the IMU by the colleagues of Lausanne, and defined as gait parameter of reference because of its combination of temporal and spatial characteristics [145], and because it is one of the most reliable predictors of mobility [146].

In the first exploratory cross-sectional study (the full manuscript is provided in Chapter 3), the distribution of gait speed from the home assessment was expressed in percentiles and amount of walking bouts, i.e., period of continuous walking. We found that analyzing maximum values of gait speed at home (i.e., best performance at home) can discriminate medication states. This suggests that maximum values of home gait speed may represent the maximum capacity of motor performance in the laboratory. We also observed that gait speed assessed at fast pace in the ON medication state in the laboratory showed the highest association with gait speed from home ($r=0.69$; $p<0.001$), and, in contrast, home gait speed showed lower correlations with normal pace walking in the laboratory ($r=0.46$; $p<0.001$). This suggests that assessing higher speeds and maximum capacity in the laboratory may give more information about PD performance in real life. Regarding circular walking, this type of gait task has also been shown to reliably represent the speed of a patient's daily-life gait [147, 148]. In contrast, gait speed assessed in the OFF medication state seemed to reflect how PD patients at home perform less than usual ($r=0.56$; $p=0.004$). Overall, these results suggest the importance of using both laboratory and home assessments to gain a comprehensive understanding of PD gait in usual environments.

The results were also implemented with a second complementary work carried out using the same PD dataset (the full paper is provided in the Appendix). In order to add useful information about the differences between supervised and unsupervised assessments, the main innovative contributions of this study was to show which type of distribution of gait speed was performed by PD patients, and the investigation of Exceptional Strides, in order to define the objective conditions under which laboratory and home tests become closer.
We found that gait speed during both laboratory and home assessment followed a bimodal distribution for almost all the patients, indicating that patients showed to have two different gait speeds. During laboratory assessment, this could be related to different gait tasks, such as straight and circular walking; during home assessment, these bimodal gait speeds may be related to indoor and outdoor walking activities [149]. This analysis demonstrated once again that clinicians can tailor clinical assessments and monitor patients by performing gait assessments under different conditions, covering a wider range of speeds which can broadly reach bimodal distributions similar to real life.
Moreover, the analysis of Exceptional Strides during real-life assessment was introduced to investigate when patients' performances reached their maximum capacity, corresponding to the ability of the patient to reach a speed equal to or greater than fast speed tested in the laboratory (i.e., the maximum capacity of the patient). This analysis showed that patients were only going beyond their maximum capacity in a very small proportion of activities of daily living, and that there was a relationship between the amount of Exceptional Strides and daily amount of L-dopa. This relationship was positive but still not statistically significant, and showed that patients with a higher number of Exceptional Strides might have taken more daily doses of medication, compared to patients without experience of Exceptional Strides during the day.
Taken together, supervised and unsupervised testing can capture complementary aspects of motor disability, particularly considering specific gait speed values as more informative when measuring patients' motor status. This may help clinicians in more accurate estimation of patient's capacity and implementation of individualized adjustments in PD therapy.

The second aim of the project was to investigate the functional impact of PNP on gait and balance, using wearable health-technology. It was achieved through the elaboration of two works. First, we elaborated a systematic review (the full manuscript is provided in Chapter 4) with two main searches: a literature search on the assessment of PNP with wearables (which included a total of 24 papers) and, separately, a search of existing reviews to provide an overview of the use of wearables in PD (13 included papers). As a result of both searches, we defined the most commonly used methodologies (type, number, and location

of wearables) and the main parameters useful for characterizing gait and balance deficits in PD-PNP. It was important to investigate the main motor characteristics of PNP and PD to guide future studies using wearable technology, in order to facilitate and simplify the use of wearables and reduce intra- and inter-operator variability. After comparing the results from both searches, we then proposed indications for assessing gait and balance in PD-PNP patients with wearable health-technology. In particular, we described three main groups of suggestions. First, the number and location of sensors on the body: the use of at least two sensors, in order to collect both PNP- and PD-specific features during gait and balance tests [39, 41]. Second, the use of more challenging gait and balance tasks: dual tasking, walking on uneven, paved trajectories, and the use of instrumented functional tests (TUG test) [150], for gait assessment. This may allow to gather all the aspects of gait deficits in PD and to reflect everyday-life conditions [151]. To perform a comprehensive balance assessment, static stance with open and closed eyes, or with the use of foams, is preferred. This strategy can be adopted to reduce the residual contribution of the proprioceptive feedback, and to understand the level of vision dependence in PNP patients. Third, particular attention should be also given to the extraction and analysis of functional parameters: gait speed, stride length and gait variability have been shown to have great association with falling [152, 153] and were the most relevant features for gait analysis in both PNP and PD populations. The total sway amplitude in both anterior-posterior (AP) and medio-lateral (ML) directions has been analyzed especially when studying postural stability in PNP [154, 155]: AP sway seems to be associated with neuropathy symptoms as a result of increased sway at the hip joint [156]. Also in PD, postural sway in both AP and ML directions has been identified as a reliable predictor of falls [151]. These parameters may be the most promising to differentiate PD patients with and without PNP.

In light of these indications, it was possible to define a personalized protocol for the assessment of PNP motor deficits in patients with PD. Subsequently, in order to investigate the functional impact of PNP in PD, we performed a cross-sectional study applying the indications of the proposed assessment on a consecutive series of PD patients (the full manuscript is provided in Chapter 5). To the best of our knowledge, this was the first study to show the impact of PNP on gait and balance in PD with the use of wearable health-technology. We included a total of 99 consecutive PD patients from the Movement Disorders' Outpatient Clinic of Centro Hospitalar Universitário do Porto (CHUP), which receives patients with PD at all stages, and with high acceptance rate. In addition, PD was accurately diagnosed by a Movement Disorders' specialist based on the UK Brain Bank criteria [157]. We first investigated the prevalence of PNP in our PD cohort, and sub-divided our participants in those with (PD-PNP) and without (PD-noPNP) diagnosis of PNP. PNP was defined using a comprehensive evaluation that included clinical, neurophysiological

and neuropathological examinations, and the PNP etiology was performed by blood tests. We defined a comprehensive evaluation of PNP based on well-defined diagnostic criteria [129]. Specifically, NCS were used to diagnose large-fiber neuropathy; clinical scales, Quantitative Sensory Testing (QST), and small nerve fiber counting from skin biopsy punches were performed to diagnose small-fiber neuropathy [128]. This allowed to understand the prevalence and impact of PNP in PD with no restriction based on PNP types and etiology, and based on rigorous and specific diagnostic criteria. We found an overall prevalence of 40.4% of PNP in the PD cohort, consistent with previously reported prevalence [70, 158]. We also showed that small-fiber neuropathy was more frequent than large-fiber neuropathy (70% of the PD-PNP cohort), and this frequency was comparable to previous reports [83, 159].

Gait and balance were assessed using a set of IMUs (Hasomed GmbH, Germany) during both OFF and ON medication states, following the proposed indications above. We found that PNP had a functional impact on both gait and balance. During gait tasks, PD-PNP patients presented significant slower gait speed, shorter stride length and smaller toe-off angles of the foot, compared with PD patients without PNP. We observed a more cautious gait in PD-PNP patients, where the contribution of neuropathic motor, sensory and proprioceptive impairments may interfere during the gait task, as also confirmed by previous studies on PNP patients [152, 160] and by a preliminary work on PD-PNP patients [144]. We also observed gait deficits during several other gait tasks (such as circular walking and turns), which for the first time validated the consistency of our results, and opened to the introduction of more ecological functional assessments for this subset of patients.

Overall, our findings confirmed that, in PD-PNP, the loss of somatosensory function and the affected neuromuscular control system may limit the capacity to respond to environmental influences during walking in both automatic and challenging tasks, and, as result, this significantly affects mobility of PD patients.

PNP also had a significant impact on balance. Postural instability was most evident during more challenging tasks, i.e. static stance on a foam with closed eyes, where the PD-PNP cohort showed more compromised balance parameters. Reduced proprioception, leading to increased dependence on vision, may cause compensatory problems during balance maintenance. It was also shown that these deficits were more frequent in the AP direction, confirming the theory that PNP may predominantly show a hip strategy (increased motion in the hip joint) to compensate for the balance deficits [155, 161].

Finally, we found significant effects of PNP on balance during OFF medication state, suggesting that optimization of dopaminergic therapy may have a relevant effect on balance in PD-PNP patients.

Overall, these findings demonstrated the importance of assessing PNP in PD, especially with regard to gait and balance complications. The use of wearable health-technology, with a customized assessment protocol, allowed for more reliable and consistent results and may pave the way for a more accurate monitoring and stratification of patients.

Finally, the third aim of the project was to develop a new method for small nerve fibers counting. It was achieved by developing a novel method of automated quantification of small nerve fibers, in collaboration with researchers from the Advanced Light Microscopy (ALM) platform of the Instituto de Investigação e Inovação em Saúde da Universidade do Porto, i3s (the full manuscript is provided in Chapter 6). Although quantification of small nerve fiber in the skin is considered an excellent method to investigate small-fiber neuropathy, manual quantitative fiber determination is prone to high variability [162]. Major reasons may concern difficulties in visualizing nerve fibers or loss of definition and fluorescence signal, especially if the analysis is repeated over time, which were also confirmed during the evaluation of small-fiber neuropathy in our PD population. For this reason, a total of 60 skin biopsy specimens from our pool of patients were subsequently used to develop a preliminary automated counting approach. We processed microscope images in their maximum projection, and we automatically defined the intradermal line. Then, an automated algorithm counting small nerve fibers passing the intradermal line was developed. We finally compared the new automated method with manual counting, performed by three independent observers on the same samples, and found a moderate-to-high correlation ($r=0.651$; $p<0.001$) between the two counting methods. With a preliminary normalization of values by 0.8, we demonstrated its possible applicability in clinical settings and for the diagnosis of small-fiber neuropathy.

One of the novelties of this method consisted in providing a new approach for image digitization: for the first time we proposed a systematic and automated identification of the intradermal line and, as consequence, a consistent reduction of variability among observers. A second novel contribution of this work was the complete automaticity of the algorithm in fluorescence images acquired with widefield microscopes, allowing not to waste time during the counting process, and to reliably identify small nerve fibers. An advantage of this method was also the use of a free and open-source software, allowing its potential applicability for further quantification analysis in different settings [163]. We recommended using this method, which is still in its preliminary stages, as a complementary approach to the classic manual nerve counting, with the goal of increasing diagnostic efficacy of small-nerve fiber neuropathy.

CHAPTER 8.
CONCLUDING REMARKS

The use of wearable health-technology has helped clinicians and researchers for decades to obtain unbiased and objective measurements. Testing gait under a variety of conditions can capture and gather complementary aspects of motor disability, and in particular, unsupervised measures can add information to clinical evaluation and patient management. Methodologically, we offered a useful tool for clinicians: we highlighted which specific laboratory tests can best represent gait at home, and thus the possibility of remotely assessing PD motor function in their domestic environment, based on their medication status. On the other hand, we defined which functional tests are most indicative of patients' performance in daily-life, helping clinicians decide which supervised tests to perform as needed. By comparing laboratory-capacity and everyday-performance, the proposed preliminary analysis allowed to monitor the effect of medications during daily activities, control medication intake, and adjust treatments according to daily motor function. Further studies with larger sample sizes and several days of measurements are still needed to capture a more granular picture of daily mobility.

Technology-based devices have also been used to investigate other aspects of PD, particularly the impact of the peripheral nervous system on gait and balance. Wearables, with a customized assessment protocol, have been successfully shown to be of great advantage for the assessment of PNP in PD, as they allowed for more ecological, faster and easier evaluations, and for the detection of minimal changes that would have been poorly perceived with other tools. Accurate quantification of several parameters may also open perspectives for establishing new cut-offs to characterize gait and balance in PD-PNP patients, and to plan personalized treatments. Motor complications caused by PNP can be delayed with the support of specific physical therapy exercises, such as balance and coordination exercises, or sensory feedback techniques (of tactile, vibratory, temperature systems), whose effects may successfully slow motor impairments and prevent falls.
More generally, our findings support the importance of a broader investigation of PNP in PD. Further studies would be needed to confirm these results, in terms of sample size with multi-centric study designs, and follow-up studies to understand the progress of PNP on quality of life. The effect of PNP could also be studied in relation to falls. Another important next step would be to extend PD-PNP functional assessment with wearables in unsupervised conditions, such as longer assessments in daily-life settings, in order to investigate the progression of PD-PNP motor function in daily-life with a broader snapshot of the clinical situation.

Regarding the clinical, neurophysiological and neuropathological assessment of PNP in PD, we performed a comprehensive unrestricted evaluation of PNP types. This allowed to investigate the overall effect of PNP in PD and to highlight the relevance for its evaluation in clinical routine. In addition, the new automated counting method can be considered as a promising preliminary step to detect small nerve fibers in skin biopsies. This method has accelerated and simplified fiber quantification, however, further analysis with larger sample size and different patient populations are needed to test and adjust the method for its use in both clinical and research settings. Promising next steps can also be implemented to characterize small nerve fibers with investigation of other relevant parameters, such as fiber length, arborizations and the nerval surface of action. This type of analysis could improve the stratification of small-nerve fiber neuropathy, increase the assessment of its progress over time, and ultimately increase the sensitivity and specificity of diagnosis.

Improving the investigation of PD with the use of new technologies, monitoring PD motor function in different environments, and studying PD features and complications such as PNP and its functional aspects, may allow for more accurate patient stratification, personalized interventions, and treatment optimization, with the aim of preventing fall risk and increasing quality of life.

CHAPTER 9.

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APPENDIX

The Appendix was adapted from the published work:

Atrsaei A, **Corrà MF**, Dadashi F, Vila-Chã N, Maia L, Mariani B, Maetzler W, Aminian K. Gait speed in clinical and daily living assessments in Parkinson's disease patients: performance versus capacity. NPJ Parkinsons Dis. 2021 Mar 5;7(1):24. doi: 10.1038/s41531-021-00171-0.
Full Open Access publication.

ABSTRACT: Gait speed often referred as the sixth vital sign is the most powerful biomarker of mobility. While a clinical setting allows the estimation of gait speed under controlled conditions that present functional capacity, gait speed in real-life conditions provides the actual performance of the patient. The goal of this study was to investigate objectively under what conditions during daily activities, patients perform as well as or better than in the clinic. To this end, we recruited 27 Parkinson's disease (PD) patients and measured their gait speed by inertial measurement units through several walking tests in the clinic as well as their daily activities at home. By fitting a bimodal Gaussian model to their gait speed distribution, we found that on average, patients had similar modes in the clinic and during daily activities. Furthermore, we observed that the number of medication doses taken throughout the day had a moderate correlation with the difference between clinic and home. Performing a cycle-by cycle analysis on gait speed during the home assessment, overall only about 3% of the strides had equal or greater gait speeds than the patients' capacity in the clinic. These strides were during long walking bouts (>1 min) and happened before noon, around 26 min after medication intake, reaching their maximum occurrence probability 3 h after Levodopa intake. These results open the possibility of better control of medication intake in PD by considering both functional capacity and continuous monitoring of gait speed during real-life conditions.

INTRODUCTION

Motor impairments in Parkinson's disease (PD) are often characterized by tremor, postural instability, and reduced gait speed (1,2). While the cause of PD is unknown, degeneration of dopaminergic nerve cells is associated with reduced motor function and impaired movement control. Therefore, PD treatments focus on the control of motor and non-motor

symptoms using dopamine compensation, mainly with Levodopa, and surgical methods such as deep brain stimulation (3). To monitor the progression of disease and symptoms, assessment scales such as the Unified Parkinson's disease Rating Scale (UPDRS) are being used widely by clinicians. Although these scales have been shown to have reliable clinometric characteristics (4), they cannot be obtained continuously and are dependent on the rater (5,6). More objective assessments can include timed tests in the lab in which gait speed can be calculated by measuring the time taken to traverse a predefined distance by stop-watch, e.g., 20-m walk test. With inertial measurement units (IMUs), gait parameters can be obtained accurately providing objective outcome measures (7–11). Based on the IMU signals or derived gait parameters, one can classify early PD (12), investigate subtle differences among PD patients (13), predict freezing of gait (14,15), monitor PD symptoms5, and the Levodopa response (16,17) in long-term daily activities. Among various gait parameters, gait speed is often considered as the sixth vital sign (18) and has been shown to be a reliable measure in diagnosis (19) and a marker of functional decline (20,21). As this parameter contains both spatial, i.e., stride length, and temporal, i.e., gait cycle time, aspects of gait, it has a strong discriminative power among patient populations (12). Being wearable, IMUs allow gait to be assessed in both clinical and domestic environments. However, as the International Classification of Functioning Disability and Health (ICF) model suggests, there is a difference between the assessments performed in the clinic which reflects functional capacity and the assessments performed during daily activities, which are more indicative of the actual performance of the individuals (22). For instance, it has been shown that during daily activities, gait speed can decrease by 30% compared to the clinic in PD patients (23). A basic explanation for this different behaviour is that mobility is not only affected by the sensorimotor system but also by psychological factors (24–27). Patients are more focused on the task and try to achieve better results in the presence of a clinician than during their actual performance in everyday life (5). Moreover, the context of the environment is different at home or outdoor where there are multiple obstacles and more complexity compared to the clinical setting (27,28) . Therefore, unsupervised assessments at home can provide additional information through long-term monitoring (29). Furthermore, it would also be possible to capture rare incidents such as falls or stage before an injury which may not be measurable during a clinical visit. Hence, domestic and clinical assessments can be considered as associated but separate domains of physical function (30). Recent studies have been trying to discover the associations between clinical and home assessments. In a group of PD patients, gait and postural transition parameters were evaluated at the clinic and home (23). It was observed that no significant correlation between clinical and home measurements exists for the patients, even for the same parameter. This study was limited in a sense as for the assessments

performed at home, the wide distribution of parameters such as gait speed was condensed to an average value. As a consequence, the large variety of gait speed at home was neglected. It has been shown that the extreme values of gait or balance parameters of home-based monitoring are more closely associated with the laboratory-based measurements (31–33). In a study, it was observed that the differences between PD patients and healthy older adults become more evident during daily living conditions because of the reduced attentional input in a real-life setting (34). However, for some parameters such as gait speed, it has been shown that during free-living conditions, only longer walking bouts could distinguish the two populations. The turning parameters have been also studied in PD patients with and without risk of falls (35). The results of this study suggested that fear of falls affects the turning behavior of the patients differently in the clinic and at home. The association of the laboratory and home-based measurements with conventional clinical assessments, e.g., the UPDRS, has been also studied. In a large group of PD patients, the authors showed that 46% of the UPDRS variance was explained by the demographic data, clinical and home assessments. From this portion, most of the variance (62%) was explained by daily living measurements (36). These studies have revealed that there is a difference between the clinical and home assessments even for the same parameter (27). The previous studies are mostly based on correlation analysis that showed the association and the difference between clinical and home measurements. Yet, the relationship between these two assessments is not fully understood. Previous studies have not shown under what conditions these differences between clinic and home are minor. Knowing these conditions, clinicians can have a better estimate of how much extent patients' capacity is being used in real-life. Therefore, in this study, we aimed towards investigating the conditions in which the clinical and home assessments become closer. More specifically, we focused on the gait speed and we have answered the following two research questions:

(1) Do patients with PD have the same preferred gait speed at the clinic and home?

(2) Under what condition does the PD patient performance measured by gait speed in free-living conditions reach the capacity measured by gait speed in the clinic?

The novelty of this study is the way we quantified gait speed distribution particularly, during daily activities in PD patients. In previous studies, the distribution has been mostly condensed to one mean and standard deviation values limiting the information we can get from this wide distribution. In this study, by including several walking tests in the clinic rather than a single gait test, we investigated the hypothesis of a bimodal gait speed distribution during both clinical and home assessments. Moreover, we have shown that how the medication state, the time of the day, and the duration of walking bouts can contribute to the difference between capacity and performance. This information can provide a better

understanding of the relationship between medication intake and the resulting increase in performance at home compared to the patients' capacity.

METHODS

Participants and study design

A total of 27 participants (11 females, 16 males) diagnosed with PD based on the UK Brain Bank criteria50 were included in the study. Measurements were taken from distinct individuals. Information about demographic data and patients' characteristics was collected from the participants (age: 70 ± 7.7 years, H&Y stage median of 2, disease duration of 7 ± 5 years, the age of disease onset: 63 ± 8.2). UPDRS including the subscales of UPDRS-II and III was obtained during both ON and OFF medication states by a clinician that was not blinded to the medication status of the patients (UPDRS II of 5.6 ± 4.5 during ON medication and 8 ± 5.9 during OFF medication, UPDRS III of 14.3 ± 10 during ON medication and 25 ± 11.8 during OFF medication). The exclusion criteria were being older than 90 years, suffering from dementia or mobility-related health problems other than PD, the inability to walk consecutively for 20 m, and having a difference of <2 between ON and OFF states in the UPDRS-III to take into account minimal clinically significant difference (51). The study was approved by the institutional review board of Centro Hospitalar Universitário do Porto (Porto, Portugal) and was performed in agreement with the WMA Declaration of Helsinki's Ethical Principles for Medical Research Involving Human Subjects (52). Written informed consent was collected from all the patients before their participation.

Clinical assessments

Patients were evaluated first at OFF state which occurred at least 12 h after their last medication intake. The patients were equipped with RehaGait (Hasomed GmbH, DE) with IMUs on each foot. After at least one hour from their medication intake, patients were considered to be in their ON medication state and were evaluated again. During each medication state, they were asked to perform a 20-m straight walk test at a convenient and fast speed as well as circular walking tests (1080° around a circle) at both left and right directions. However, due to the difficulties of the patients to complete the straight walking test at fast speed, this test was skipped during OFF. The clinical gait assessments are summarized in Table 3.

Home assessment

The next day, patients came to the hospital again around 9:00 in the morning to be equipped with Physilog® 5 (Gait Up, CH) IMUs on the right foot. The patients were asked to go back home and perform their daily routine activities for one day. It should be noted that patients were allowed to go outside the home and perform their usual daily activities. Therefore, “home assessment” can also include daily activities that had been done outside their living space. The sensors were programmed to start recording automatically at 10:00 for 12 h, i.e., until 22:00. The patients recorded the time of their medication intake in a diary. Based on their diary, we have assumed and defined the ON state periods as starting one hour after taking the medication and lasting for a period of two hours and the OFF state periods starting half an hour before taking the medication and lasting for a period of one hour (53,54).

Gait speed and walking bout extraction

For all of the clinical gait tests mentioned in Table 3, the raw data of gyroscope and accelerometer from both of the feet were used. To have a more steady-state gait, the first and last two strides were discarded. With a previously validated algorithm (45), gait speed was obtained for each gait cycle by the right foot IMU. Since each of the clinical tests (Table 3) contained only one walking bout, no analysis regarding the detection of walking bouts was made as opposed to the home assessment. In addition to the gait speed for each gait cycle, the mean value of the gait speed throughout the test was also calculated. For home assessments, first, the walking bouts were detected using the angular velocity signal (55). To have enough steps within each walking bout, the walking bouts that had a duration of <15 s were discarded. This was done to prevent detecting other movements than gait that can impact our analysis wrongly. Furthermore, removing very short walking bouts let us have a more steady-state gait during daily activities. Next, within each walking bout, gait speed was calculated for each gait cycle (45). Gait cycles with a speed of <0.2 m/s were discarded as these could potentially be a break. Walking bouts were divided into short (duration between 15 and 30 s), medium (duration between 30 and 60 s), and long (duration of more than 60 s) bouts.

Distribution of gait speed at the clinic and home

To obtain a distribution for the gait speed, all the gait cycles were considered for each clinical and home setting. There is some evidence in the literature for a bimodal Gaussian distribution during daily-life gait speed (31) and cadence (42). As in the current study we had performed several clinical gait tests in various conditions, we considered the bimodal distribution f (x) for each of the clinical and home assessments, in which x is the gait speed distribution, c1 and c2 determine the amplitude, μ1 and μ2 are the means presenting the

preferred lower and higher gait speed (31), and σ1 and σ2 are the standard deviations from each of the means.

$$f(x) = c_1 \exp\left(-\frac{1}{2}\left(\frac{x-\mu_1}{\sigma_1}\right)^2\right) + c_2 \exp\left(-\frac{1}{2}\left(\frac{x-\mu_2}{\sigma_2}\right)^2\right)$$

MATLAB's fitgmdist function was used to fit the Gaussian models. Ashman's D was calculated to quantify the fitting quality. A value of >2 is indicative of a bimodal distribution (56). The two means and standard deviations were compared together between clinical and home assessments using a two-sided t-test for normally distributed data or Wilcoxon rank sum test for data that did not follow a normal distribution. One-sample Kolmogorov–Smirnov test was used to test for the normality of data. Pearson's correlation coefficient with the criteria given in (57) for low, moderate, and high correlations was also obtained. To observe the differences between the preferred gait speeds at clinic (μ1;clinic, μ2;clinic) and at home (μ1;home, μ2;home), we defined two parameters Δμ1 and Δμ2 that represent the percentage of difference between clinic and home for μ1 and μ2, respectively.

$$\Delta_{\mu_1} = \frac{2\left(\mu_{1,\text{clinic}} - \mu_{1,\text{home}}\right)}{\mu_{1,\text{clinic}} + \mu_{1,\text{home}}} \times 100$$

$$\Delta_{\mu_2} = \frac{2\left(\mu_{2,\text{clinic}} - \mu_{2,\text{home}}\right)}{\mu_{2,\text{clinic}} + \mu_{2,\text{home}}} \times 100$$

We obtained Pearson's correlation coefficient between number of doses and Δμ1 and Δμ2 considering all the patients. Furthermore, the cumulative distribution function of gait speed at the clinic (CDFclinic) as well as home (CDFhome) were determined for each patient. Receiver operating characteristic (ROC) curve was obtained for each patient by considering CDFhome as the x axis and CDFclinic as the y axis. Finally, for each patient, the area under the ROC curve (AUC) was calculated. An AUC value close to 0.5 means that the clinical and home assessments have the same gait speed distribution while a value closer to 0 (or 1) means that the probability of having a gait speed less than a specific value is higher at home (or in the clinic).

Capacity vs. performance (Exceptional Strides)

For each patient, their average gait speed during the 20-m walk test with fast speed (at ON medication) was obtained and taken as their capacity (Vc). To investigate when patients reach their capacity Vc or go beyond it during daily activities, for each stride k, its gait speed (Vh;k) was compared to Vc and if it was greater or equal than Vc, it was marked as an Exceptional Stride and the following information was extracted for that stride:

● Time of occurrence (tk)
● Its time difference compared to the last medication intake (tk - tc)
● Whether it happened during ON state or OFF state (MEDk)
● The duration of its corresponding walking bout (TWB;k)
● Whether it happened during short, medium, or long walking bout
(WBk)
● Its gait speed difference compared to Vc (Vh;k - Vc)

To correct for measurement errors, a threshold of 0.1 m/s was used when comparing Vh;k and Vc to obtain the Exceptional Strides. The impact of the status of PD on the percentage of Exceptional Strides over the total number of strides for each patient was examined. We calculated the correlation coefficient between the amount of Exceptional Strides and UPDRS-III (at OFF medication) as well as the correlation coefficient between the amount of Exceptional Strides and number of medication intakes during the day.

RESULTS

Distribution of gait speed at the clinic and home

The mean gait speed during clinical assessments was compared to the distribution of the gait speed at home for all the patients (Fig. 1). The average value of the 20-m walk test with fast speed, considered as the capacity of the patients, were near to or even higher than the maximum values of the gait speed measured at home. Furthermore, the average value of the circular walking tests was lower than the other clinical assessments. The average duration of the straight walking tests for all the patients was 18.5 ± 3.8 s.

For a typical patient, the histogram of the gait speed as the probability density function distribution is shown in Fig. 2 along with the fitted Gaussian mixture models during daily activities and all the clinical assessments. The bimodal distribution of the gait speed at both home and clinic can be inferred from this figure. The patient had two preferred gait speeds, a lower (μ1) and a higher one (μ2) during both clinical and home assessments. The standard deviations from these two preferred speeds were denoted by σ1 and σ2. For this specific patient, the preferred gait speeds at home (0.44 and 0.83 m/s) were close to the preferred speeds at the clinic (0.45 and 0.90 m/s).

For the clinical measurements, the distribution was also shown colored with the type of the test. The circular walking tests constructed the left part of the distribution and the straight walking tests constructed the right part of the distribution.

To evaluate the existence of bimodal Gaussian distribution in the whole group of the patients, for each patient their gait speed distributions in the clinic and at home were normalized by the 95th percentile of the respective distribution (Vc;95 for clinical

assessment, Vh;95 for home assessment). The gait speed distributions in both clinic and daily activity are depicted by considering all patients congregated (Fig. 3). During the clinical assessment, the circular walking tests lay more on the left of the distribution, the straight walking tests with convenient speed were in the middle and the fast walking tests were at the right of the distribution.

The fitting quality of the bimodal Gaussian distribution estimated by Ashman's D value was higher than 2 for all the clinical assessments. However, for three patients (P5, P8, and P26), this value was below 2 during their home assessment, meaning that there was not a clear separation between the modes of gait speed distribution at home. For all the remaining patients, the means (μ1 and μ2) and standard deviations (σ1 and σ2) were compared between the clinic and home with the Wilcoxon rank-sum test (Table 1).

No significant difference was observed between the means (μ1 and μ2) and the standard deviation corresponding to the higher preferred gait speed (σ2) between the clinical and home assessments. However, the standard deviation corresponding to the lower preferred gait speed (σ1) was significantly higher at home compared to the clinic (p-value < 0.001). These results show that the patients had on average the same preferred gait speeds at the clinic and at home with the same deviation from the higher preferred gait speed. However, their gait speed variation around the lower preferred gait speed was significantly higher during daily activities. A moderate correlation was found for the higher preferred gait speed (μ2) between the clinic and home (ρ = 0.61, p-value = 0.0015, 95% confidence interval: 0.28: 0.81). The correlation between the lower preferred gait speed (μ1) was also moderate (ρ . 0:52, p-value = 0.0084, 95% confidence interval: 0.15: 0.77). No significant correlation was found for the standard deviations (σ1: ρ = 0.06, p-value = 0.7897, 95% confidence interval: −0.34: 0.46 and σ2: ρ -0.02, p-value = 0.8898, 95% confidence interval −0.32: 0.48).

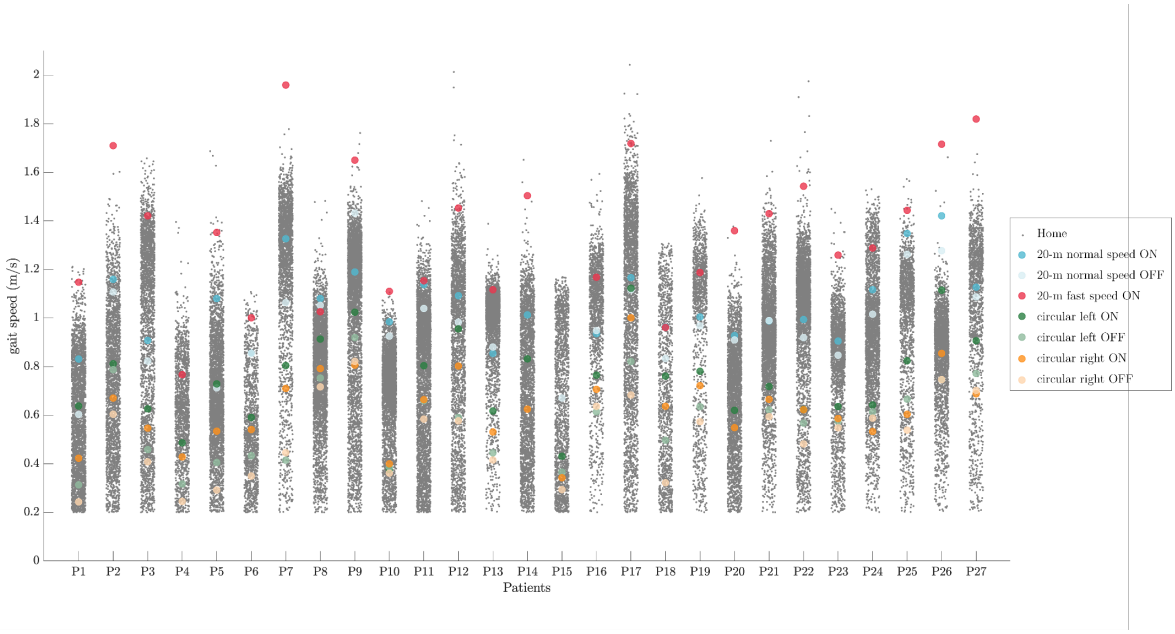

Figure 1. Distribution of gait speed at home and the average values of the gait speed for the clinical assessments for each patient. For each patient, the average gait speed during the 20-m walking test was considered as their capacity.

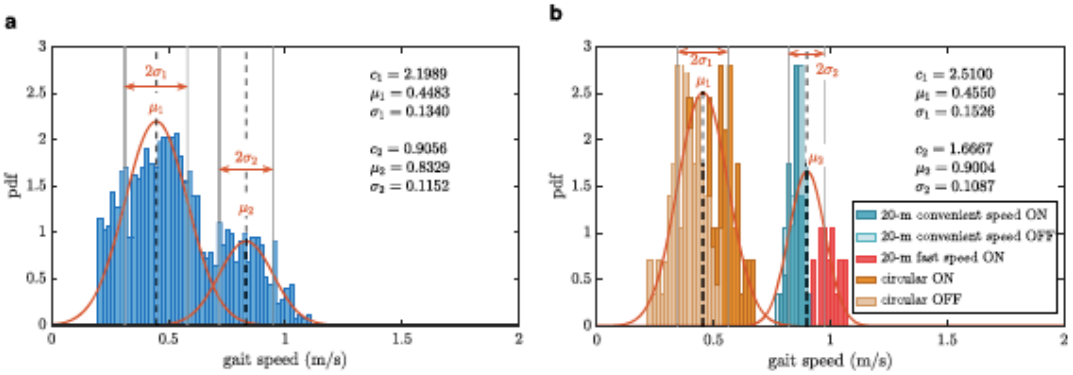

Figure. 2 An example of the gait speed probability density function for one of the patients (P6). The distribution is shown at (a) home and (b) at the clinic.

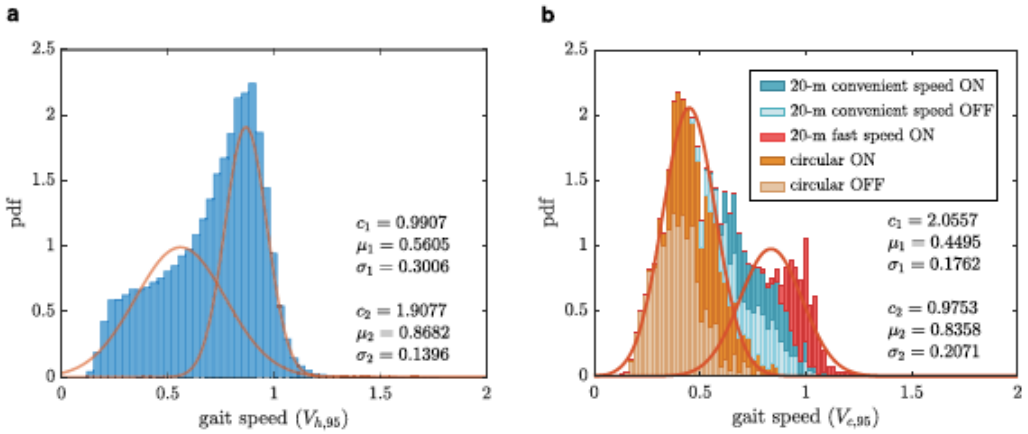

Figure 3. The gait speed probability density function (pdf) for all the patients together. The distribution is shown at (a) home normalized by $V_{h,95}$ and (b) the clinic normalized by $V_{c,95}$, the red fitted curves are the first and second terms of the bimodal Gaussian distribution.

Table 1. Comparison of the preferred gait speeds along with their corresponding deviations between clinic and home, the significance level was set to 0.05

	Home (m/s)		Clinic (m/s)		Comparison	Correlation	
	Median	IQR	Median	IQR	p-value	ρ	p-value
μ_1	0.47	[0.44 , 0.73]	0.63	[0.47 , 0.71]	0.3173	0.52	**0.0084***
μ_2	1.00	[0.88 , 1.14]	1.02	[0.90 , 1.41]	0.5028	0.61	**0.0015***
σ_1	0.17	[0.13 , 0.26]	0.08	[0.07 , 0.15]	**<0.001***	0.06	0.7897
σ_2	0.14	[0.11 , 0.16]	0.14	[0.08 , 0.23]	0.6725	-0.02	0.8898

Δμ1and Δμ2 as the percentage of the differences for preferred gait speeds between clinic and home were shown in Fig. 4a. The median values are 6% and 7%, for Δμ1and Δμ2 , respectively. The 25th and 75th percentiles are <23%, and the upper and lower adjacent values can reach up to 60%. The AUC values that present the similarity of the cumulative distribution functions of clinic and home were shown in Fig. 4b. The median value was obtained as 0.64 and the 25th and 75th percentiles as 0.51 and 0.68, respectively. The correlation between the number of medication doses taken during the course of data recording and Δμ1 was ρ = -0.19 (p-value = 0.3649, 95% confidence interval: −0.55: 0.23). The correlation between number of medication intakes and Δμ2 was ρ = -0.50 (p-value = 0.0126, 95% confidence interval: −0.75: −0.12). Plotting the number of medication doses intake versus Δμ1 and Δμ2 in Fig. 5 revealed that patients with a higher number of Levodopa intakes during daily activities performed faster at home (Δμ2<0) while patients with a lower number of Levodopa intakes performed faster in the clinic (Δμ2>0).

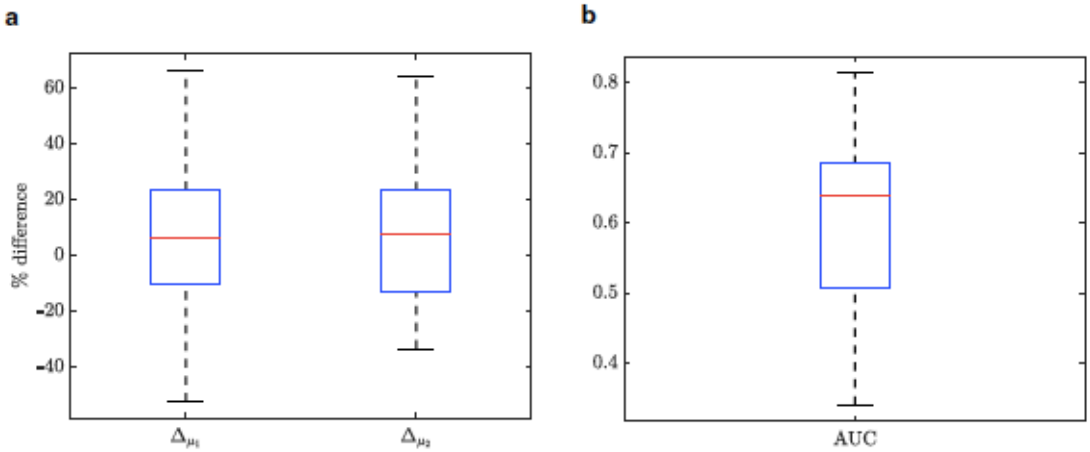

Figure 4. The boxplots comparing the gait speed distribution between the clinic and home for all the patients. (a) The percentage of the difference between clinic and home for preferred gait speeds μ1and μ2, **(b)** the area under the ROC curve (AUC) of CDF-clinic versus CDF-home: Center line: median; box limits: upper and lower quartiles; whiskers: 1.5 x interquartile range.

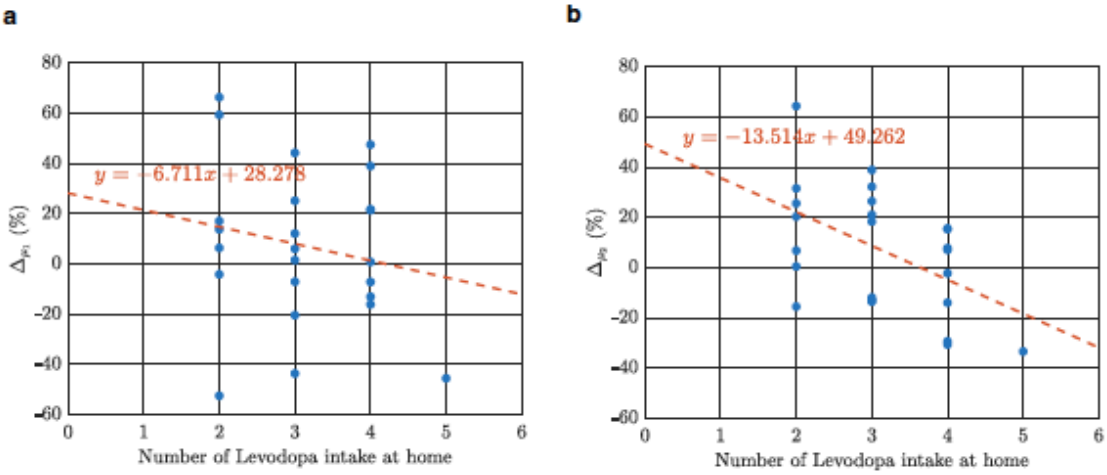

Figure 5. The relationship between the number of medication doses taken during the interval of data recording in home assessment. This linear relationship is shown for **(a)** Δμ1 and **(b)** Δμ2.

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Exceptional Strides

Regarding the Exceptional Strides, the information concerning one Exceptional Stride k (section II-F) as an example, is shown for one of the patients (Fig. 6). This specific Exceptional Stride happened 0.34 h (20.4 min) after the last Levodopa intake at 17:00. Therefore, it happened during the predefined OFF state. Furthermore, this stride belonged to a walking bout with a length of 84.8 s considered as a long walking bout. The gait speed of this stride was 0.01 m/s higher than the patient's capacity (Vc). Out of 27 patients, 3 patients did not have any Exceptional Stride in their home assessment (P2, P7, and P27). Furthermore, for one of the patients (P15), no data was present from their 20-m straight walk test with fast speed as depicted in Fig. 1. Stacking the data from the remaining 23 patients together, the aforementioned parameters were given in Table 2. It can be observed that a median of 104 Exceptional Strides existed from all the 23 patients (see Table 2). For each patient, the number of their Exceptional Strides was normalized by their total number of strides. It can be seen that 3.4% of their gait cycles had a speed higher than or equal to their capacity at the clinic. A negative but insignificant trend was observed between the amount of Exceptional Strides and UPDRS-III (ρ = -0.10 p-value = 0.6344, 95% confidence interval: −0.47: 0.30). Moreover, a positive but insignificant relationship was found between the amount of Exceptional Strides and number of Levodopa intakes (ρ = 0.17 p-value = 0.4144, 95% confidence interval: −0.23: 0.52). Exceptional Strides occurred at a median of

11.74 h or a bit before noon (11:44). The 3D histogram plot for the time of occurrence of the Exceptional Strides (tk) as well as their time difference with regard to their previous medication intake (tk - tc) is shown in Fig. 7. In this figure, the yellow bar demonstrates the highest peak of the Exceptional Strides that occurred around 10:00 to 10:30 and had a time difference of ~2 h with their previous medication intake. Therefore, they correspond to the medication doses taken around 8:00 to 8:30. Other peaks can be observed around 12:00 and 17:30. Regarding the time difference between the Exceptional Strides and their corresponding last medication intake, the median value was 2.80 h which states that most of the Exceptional Strides happened 2.80 h after taking Levodopa. The probability distribution function (pdf) of the time differences were plotted in Fig. 8 along with the fitted kernel density smoothening function. Two peaks can be distinguished from the kernel smoothening function at 0.44 and 2.97 h. This implies that around half an hour and three hours after taking the medication, there is a high probability of having a gait speed equal or greater than the capacity at the clinic. Moreover, a sharp drop can be observed at ~1 h after taking the medication. Furthermore, the probability of having an Exceptional Stride during ON state was higher than during OFF state (Table 2). While the median of the walking bout duration in which the Exceptional Strides had occurred (TWB;k) was 46.17 s, most of the Exceptional Strides happened in long walking bouts, i.e., walking bouts with a duration of more than 60 s. 89.5% of the Exceptional Strides belonged to long walking bouts while this amount was reduced to 7.0% and 0.9% in medium and short walking bouts, respectively. Finally, the median difference between the gait speed of the Exceptional Strides and the capacity (Vh;k - Vc) was obtained as −0.02 m/s (Table 2).

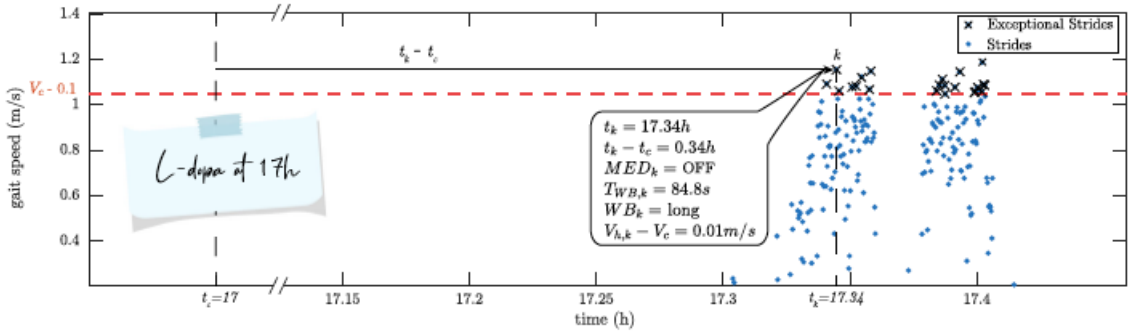

Figure 6. The information extracted for Exceptional Stride k for one of the patients as an example. Each blue dot shows the gait speed of a gait cycle at a specific time of the day during daily activities. This patient took Levodopa at time tc = 17h. Vc is the capacity of the patient, i.e., gait speed during fast walking test in the clinic. The Exceptional Strides have marked with black crosses. k is one example of the Exceptional Strides with the information extracted according to section II-F. No walking with a duration of more than 15 s occurred after 17 h and before 17.3 h.

Table 2. The parameters of Exceptional Strides for all the patients except P2, P7, P15, and P27.

	Median	IQR
Number of Exceptional Strides	104	[32 , 557]
Normalized number of Exceptional Strides (%)	3.36	[0.92 , 25.09]
t_k (h)	11.74	[10.57 , 14.59]
$t_k - t_c$ (h)	2.80	[2.03 , 3.42]
$MED_k = \text{ON}$ (%)	27.42	[3.54 , 75.85]
$MED_k = \text{OFF}$ (%)	3.89	[0.19 , 26.08]
$T_{WB,k}$ (s)	46.17	[26.10 , 129.39]
$WB_k = \text{short}$ (%)	0.89	[0 , 10.28]
$WB_k = \text{medium}$ (%)	6.97	[3.90 , 19.40]
$WB_k = \text{long}$ (%)	89.46	[72.81 , 95.11]
$V_{h,k} - V_c$ (m/s)	-0.02	[-0.06 , 0.04]

DISCUSSION

In this paper, we aimed to investigate under what conditions the clinical and home measurements demonstrate a close association. In previous studies, it had been proven that there are differences even for the same parameter obtained during clinical and home assessments (23,31,34,37–40). However, to the best of our knowledge, it has not been investigated under what circumstances the gap between clinical measurements and real-life daily activities becomes smaller.

Gait speed was extracted during functional tests performed at the clinic and during daily activities in real-life settings. Several walking tests were performed at the clinic during both ON and OFF states to capture different aspects of the patients' gait. During daily activities, we discarded the walking bouts with a duration of <15 s to include walking bouts with a steady-state gait speed. This value is reasonable as the duration of the straight walking tests during the clinical assessment was around 18 s making the comparison between clinic and home fairer. It was shown that the 20-m straight walking test with fast speed lay at the extreme end of the gait speed distribution at home (Fig. 1). This is in line with what has been previously reported in the literature (27,31,41). Comparing the gait speed obtained during daily activities and a 4-m walk test at the clinic in community-dwelling participants, one previous study showed that the high percentiles of the gait speed distribution at home had higher correlations with the 4-m walk test at the clinic (31).

Specifically, for three patients, i.e., participants #16, 18, and 19, their fast walking test at the clinic had relatively slower speed compared to their maximal performance at home as

there were many gait cycles with a higher speed at home (Fig. 1). While due to psychological factors people behave differently in different settings (28), we believe that some other reasons can also explain this difference. We checked the assessment data of these patients in more detail, and found that they all performed their walking tests in the clinic formally during best ON medication, i.e., about 90 min after their last Levodopa intake. Therefore, we were reassured that the protocol of the test regarding the assessment time after the medication intake was respected for these patients. Moreover, their treatment response as defined by the UPDRS-III scores (participant #16: 26 points during OFF, 20 points during ON; participant #18: 31 versus 12; participant #19: 22 versus 8) indicates good Levodopa response. Nevertheless, we believe that the effect of the medication can be different for each patient and patients can respond differently to dopaminergic medication especially concerning pharmacodynamic aspects. This, in fact, shows that home assessment can have complementary information to clinical assessment and may give us a better insight about the actual capacity of the patients. While the reasons for these differences in the clinic versus home behavior remain unclear, our study may stimulate further investigation in this area of research.

The gait speed distribution during both of the clinical assessments and daily activities followed a bimodal distribution for almost all the patients. This indicates that patients had two different preferred gait speeds. During clinical assessment, this phenomenon is because patients were assessed basically under two groups of walking tests, demanding as well as simpler ones. During home assessment, we can assume that the lower preferred gait speed is more attributed to shorter walking bouts that occur more indoors and higher preferred gait speed to the longer walking bouts that might occur more outdoors. Although we did not ask the patients to register the information about their indoor or outdoor activities, having this information could have confirmed our hypothesis. This bimodal phenomenon has been shown in previous studies for gait speed (31) and cadence (42) in community-dwelling adults during daily activities. In this study, we have confirmed this phenomenon in PD patients during daily living measurements. The advantage of such quantification of gait speed distribution is to preserve the information of this wide distribution rather than condensing it to one mean and standard deviation value.

In Fig. 2 and 3, it was shown that the circular walking tests composed the lower scales of the gait speed distribution while the straight walk tests constructed the higher gait speeds. This is not surprising as patients can have a lower gait speed in more demanding tasks. In a study on older fallers, it was shown that the gait speed obtained during dual-task walking tests corresponded better to the daily activities as opposed to the usual walking test (41). This shows that performing more demanding walking tests in the clinic can give a better view of the patients' performance at home and clinicians can adapt or choose the most

relevant clinical assessments. In other words, more demanding walking tests such as circular walk tests or dual-task tests represent the patients' lower preferred gait speed and simple walking tests such as straight walk tests represent the patients' higher preferred gait speed during daily activities.

Comparison of the bimodal distribution between clinic and home showed that patients had on average the same preferred gait speeds in both of the settings (Table 1). There was a significant difference between the two settings for the variations from the lower preferred gait speed (σ1) but not from the higher gait speed (σ2). Patients had higher variability for their lower preferred gait speed at home compared to the clinic. This can be explained by the complex context of the environment in real-life settings, e.g., turns, curved paths, obstacles, which causes people to continuously adapt their gait speed (37). However, the variations around the higher preferred gait speed (σ2) was not significantly different between real-life and clinical setting. This might be because the higher preferred gait speed expresses the capacity of the patients which might stay constant between clinic and home. This can also explain the higher correlation for the higher preferred gait speed (ρ = 0:61) between lab and home compared to the lower preferred gait speed (ρ = 0:52). Another contributing factor can be the use of different vestibular systems when we walk slowly or fast (43,44).

While the statistical test did not show a significant difference between clinic and home for the preferred gait speeds (μ1 and μ2), this lack of significance can be due to lack of power. To this end, we introduced additional parameters (Δμ1 and Δμ2) to look at the difference between clinic and home more deeply. Δμ1 and Δμ2 showed that for most of the patients, the difference between clinic and the home was <23% while there were few patients that had a larger difference of up to around 60% between clinic and home (Fig. 4a). The AUC values that were on average about 0.64 confirmed that the cumulative distribution function of gait speed in clinic and home are comparable (Fig. 4b).

The reason for this difference between clinic and the home was partly explained by the variation in PD as there was a significant and moderate correlation between the number of Levodopa intakes throughout the day and Δμ2 (Fig. 5). These results suggest that higher numbers of daily Levodopa intakes have a positive impact on the preferred walking speed at home, especially in the "capacity area" (μ2). However, we should also consider that patients with a lower number of Levodopa intakes tend to respond better during clinical assessments. Another similar but independent reasoning can be the rationale behind why certain PD patients may get a little number of daily Levodopa prescribed, e.g., because they may not be able to manage a complex medication regimen. Considering our limited sample size, whatever the reasons are for this observation, such analyses can serve as the first

steps into a better understanding of the relation between medication intakes and the difference between clinical and home assessments.

Therefore, to answer our first research question which was whether patients have the same preferred gait speed in the clinic and at home, we showed that by performing gait assessments under different conditions in the clinic, we can cover a wide range of gait speeds that can reach on average similar bimodal distribution observed in real-life. Nevertheless, daily-life measures can still provide complementary information to the clinical assessments (27,30).

To answer the second research question which was investigating the instances in which the patients' performance reaches their capacity, we introduced and detected the Exceptional Strides for each patient during real-life conditions. These strides express the ability of the patient to reach equal or greater gait speed than the fast speed in the clinic (Vc) considered as the capacity of the patients. We considered a threshold of 0.1 m/s to compensate for the measurement errors. This value can be justified by the error of the employed algorithm (around 5 cm/s) to extract gait speed as shown in45. Exceptional Strides constituted only 3.4% of the total strides of the patients (Table 2). This reveals that in very small part of daily activities patients went beyond their capacity.

Although not significant, a negative relation was found between UPDRS-III and the amount of Exceptional Strides meaning that patients with higher UPDRS-III can have a lower number of Exceptional Strides. Moreover, the positive but insignificant relation between the amount of Exceptional Strides and the number of Levodopa intakes taken during the day suggests that patients with higher amounts of Exceptional Strides might have taken a higher number of medication doses. However, more evidence with a larger dataset is needed to confirm these findings.

Histogram plot of Exceptional Strides time of occurrence (Fig. 7) showed that most of the Exceptional Strides happened before noon. This confirms the finding in the literature that PD patients with early or moderate stage of the disease have similar pattern of diurnal activity and are more active in the morning with a late morning peak46. This may be explained by being more active and having more walking bouts that occurred in the morning. These strides decreased in the afternoon reaching a minimum at 13:30 which might be due to a decrease in activity levels after lunch. Exceptional Strides increased again reaching their maximum around 17:00 in the evening which can again be due to the recovered energy before the end of the evening. Moreover, some of the patients were going to work; therefore, coming back from work can be another potential explanation to have Exceptional Strides at 17:00. However, the Exceptional Strides count was still approximately only one third compared to the morning. Having the Exceptional Strides mostly in the morning can also be due to the study design as the patients had to go back home from the hospital, therefore

they might have had more long walking bouts and consequently more Exceptional Strides in the morning.

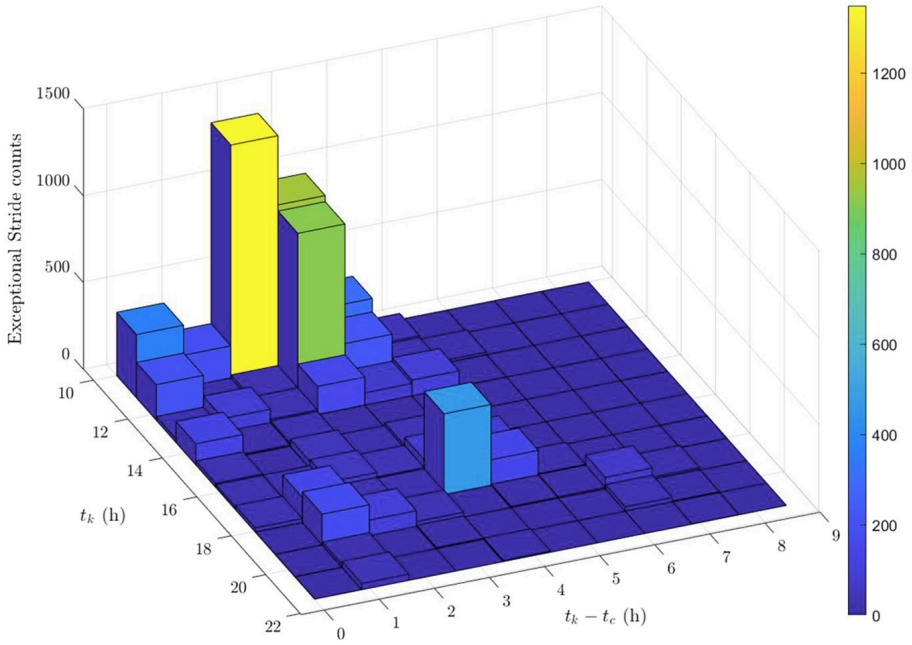

Figure 7. 3D Histogram plot of Exceptional Stride time of occurrence (tk) and their time difference from their corresponding previous medication intake (tk - tc). The yellow bar demonstrates the highest peak of the Exceptional Strides that occurred around 10:00 to 10:30 and had a time difference of ~2 h with their previous medication intake. Therefore, they correspond to the medication doses taken around 8:00 to 8:30.

The effect of Levodopa might be considered as maximum, ~3 h after taking the medication as the Exceptional Strides occurred mostly at this time (Table 2). This is in line with a previous study that presented a model for Levodopa medication effect in finger tapping tests (47). It was shown that the tapping frequency increased around 30 min after taking Levodopa and was at its maximum of around 180 min. In another study, by monitoring the stride length of the patients during daily activities, it was reported that the onset of the medication was 24 min. We have obtained almost the same value, as it can be observed in Fig. 8, there was an increase in the number of Exceptional Strides 0.44 h or 26 min after the medication intake. As expected, Exceptional Strides occurred more frequently in ON state periods compared to the OFF state periods (Table 2). Our initial assumption of ON state periods in which we considered between 1 and 3 h after taking the medication was generalized to the whole population. However, such a generalization might not be accurate for an individual patient due to different treatment responses. Moreover, the emergence of Exceptional Strides in less than half an hour for some patients (Fig. 8) might suggest that the initial assumptions for OFF state periods might not be true. Therefore, having the

information about patients' performance during daily activities and comparing it to their capacity in the clinic can provide the potential to determine and monitor the effect of Levodopa in PD patients in a personalized manner. This is again in favor of the complementary aspect of information from daily living measurements.

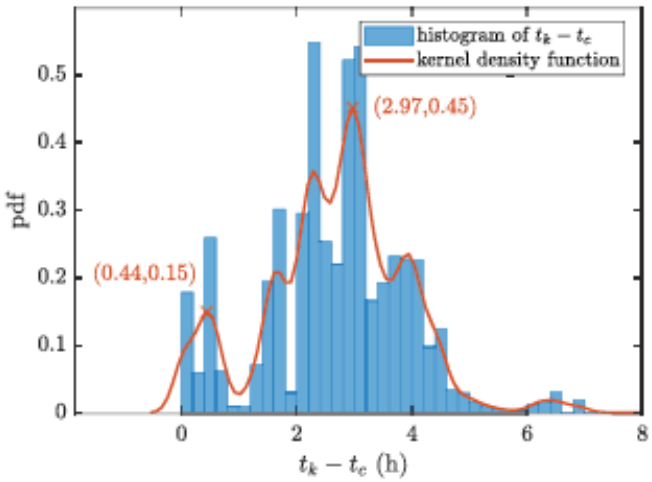

Figure. 8 The probability distribution function (pdf) of Exceptional Strides in relation to medication intake time (blue) with the fitted Gaussian mixture model (red). Two peaks can be distinguished from the kernel smoothening function at 0.44 and 2.97 h.

It was observed that the occurrence of the Exceptional Strides was hardly seen in short walking bouts as only <1% of them happened during this type of walking bout. This percentage was increased in medium and long walking bouts with long walking bouts having a large portion of the Exceptional Strides (almost 90%). This can be justified by the fact that shorter walking bouts might occur when there are obstacles in the walking path of the individuals making them pause or stop their gait. Furthermore, shorter walking bouts can occur when people are doing several daily tasks requiring more attention and as a consequence causing the reduction of gait speed. However, for longer walking bouts, people can reach a more steady-state gait speed where it can be expected that the main task of walking is less perturbed by secondary tasks as is the case in the clinical assessment (28). The importance of considering longer walking bouts to predict PD has also been shown in another study (34). It was shown that short walking bouts of <20 s cannot reveal a significant difference between the control group and PD patients' gait speed. However, as the duration of the walking bouts increases, the corresponding gait speed difference between the control and PD group becomes larger, reaching its maximum for walking bouts of longer than 2 min.

Finally, we observed that the Exceptional Strides' gait speed deviated between −0.06 and 0.04 (Table 2). Therefore, the threshold of 0.1 m/s to consider Exceptional Strides seems

reasonable as it lay outside these two values. This threshold was considered only due to the error of our gait speed estimation system. However, to take into account also the performance of the patients individually, an adaptive threshold based on each patient's gait speed range can be employed.

The main contribution of the current study was a new approach to compare clinical and home assessments, firstly, by comparing the bimodal distribution of gait speed between clinic and home, and secondly, by the Exceptional Strides. These approaches could preserve the information regarding the type of walking bouts, the medication effects, the time of the day as well as the complex distribution of gait speed that has been mostly limited in the literature to a unimodal distribution. Thanks to these two approaches, we were able to determine the conditions that lead patients to reach their capacity. In this way, the clinicians can know to what extent the patients' capacity is being used during daily activities, especially if a walking test in the clinic is not possible and patients are being monitored remotely in their domestic environment due to situations such as the COVID-19 pandemic (48). Looking specifically at the difference between the higher preferred gait speed at home and clinic (Δμ2), the 97th percentile of gait speed distribution at home (because we showed Exceptional Strides compose 3% of the gait cycles), walking bouts longer than 1 min, gait cycles happening in the morning, and gait cycles around 3 h after taking the medication has the potential to give some information about the capacity of the patients.

Moreover, to the best of our knowledge, there is no previous work investigating the effect of medication on the difference between clinical and home assessments of gait speed. The comparison of bimodal gait speed distribution between the clinic and the home was shown to have the potential to estimate the optimal number of medication doses throughout the day. Moreover, the effect of medication intake can be monitored objectively by comparing capacity and performance. This can help the clinicians to design the optimal dose of the medication for the patients. Yet more evidence in a larger dataset including healthy controls is needed to determine a meaningful relationship between the number of Exceptional Strides and the stage of PD.

The first limitation of our study was that daily activity assessments have been performed only in one day. Several days or a week could be more relevant to capture all the aspects of daily activities as people may have different amounts of activity, e.g., on weekdays and weekends (38).

Another limitation of this study was neglecting very short walking bouts, i.e., walking bouts having <15 s duration as these very short walking bouts compose most of the walking bouts during daily activities (34). These walking bouts could have improved probably the power of calculations. Nevertheless, removing those very short walking bouts made our analysis fairer and also let us obtain a more steady-state gait speed during home assessment.

We did not distinguish curved walking bouts from straight walking bouts during daily activities. An algorithm such as the one introduced by reference (49) can be employed to detect turnings during daily activities and differentiate the walking bouts during curved and straight paths. As this algorithm was designed for an IMU on the lower back, a sensor on the lower back can be useful for this purpose. Furthermore, the effect of the duration of the walking bouts on the comparison between clinic and real-life should also be studied.

Finally, in the current study, we investigated the circumstances in which the clinical and daily living measurements were more associated together. Although the findings can help the clinicians to know which tests in the clinic are better representative of daily living measurements, or vice versa, which conditions during daily living are better indicative of capacity in the lab, they do not concern about the information that is not mutual between clinic and home.

To conclude, this study presented new insights to investigate when daily activity performance reaches the functional capacity as measured in the clinic. By collecting all walking bouts and estimating their speed, we found that PD patients had a bimodal gait speed distribution during real-life conditions with on average similar modes as the gait tests performed in clinic during various conditions and speeds. Further analysis at stride level showed a low percentage of strides (~3%) had a gait speed equal or greater than the maximum speed in clinic considered as patients' capacity. These strides, termed as Exceptional Strides, happened mostly before noon, during ON state, and walking bouts with at least 1-min duration. There was an increase in the number of Exceptional Strides starting 26 min after medication intake reaching the maximum at 3 h. It was also concluded that by comparing the capacity and performance, one can monitor the effect of medication during daily activities and possibly adapt it to reach a gait speed closer to that of the capacity more frequently. Future research is however necessary to determine the meaningful relationship between the number of Exceptional Strides and the progression of the disease as well as the amount of Levodopa intake.

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