STUDY PROTOCOL

Relationship Between Cognitive Function, Trail-Walking Test Performance, and Freezing of Gait in Parkinson's Disease

1. Study Overview and Rationale

Parkinson's disease (PD) is a progressive neurodegenerative disorder associated with motor and non-motor symptoms that can substantially affect gait and mobility. Freezing of gait (FOG) is a particularly disabling motor symptom characterized by brief episodes of absent or markedly reduced forward progression of the feet despite the intention to walk. FOG is frequently associated with impaired executive function and difficulties in performing motor and cognitive tasks simultaneously.

The Trail Walking Test (TWT) is a motor-cognitive dual-task paradigm adapted from the neuropsychological Trail Making Test (TMT). It combines walking with directional changes and cognitive processing, potentially providing additional information about motor-cognitive interference during gait in individuals with PD.

The present study will investigate the relationship between TWT performance, executive function, and FOG severity in individuals with PD. An age-matched healthy control group will be included to provide a reference for comparing motor-cognitive walking performance between individuals with PD and healthy adults.

The assessment will combine clinical evaluation, neuropsychological testing, standard gait assessment under single-task and dual-task conditions, TWT performance assessment, and a structured evaluation of FOG. Wearable inertial measurement unit (IMU) sensors will be used to record gait-related parameters during all walking tasks.

2. Study Objectives

The study aims to:

1. Determine whether TWT performance provides additional information about cognitive performance beyond standard single-task and dual-task gait assessments.
2. Investigate the relationship between TWT performance and executive function.
3. Investigate the relationship between TWT performance and FOG severity.
4. Compare TWT and gait performance between individuals with PD and age-matched healthy controls.

3. Study Design and Setting

This is an observational, cross-sectional study involving two groups: individuals with Parkinson's disease (PD) and an age-matched healthy control group.

Patients with PD will be recruited from the outpatient services of the Department of Neurology, First Faculty of Medicine, Charles University and General University Hospital in Prague.

All patients with PD will undergo assessment in the medication-ON (Med-ON) state, defined as 60–90 minutes after administration of their usual levodopa dose.

All assessments will be performed during a single study visit according to a predefined testing protocol. No experimental treatment or modification of participants' usual medication regimen will be introduced as part of the study.

The expected duration of the study visit will be approximately 60–90 minutes.

Planned study period: December 2026–December 2027.

4. Study Population

4.1. Parkinson's Disease Group

The PD group will consist of adult patients diagnosed with idiopathic PD who experience freezing of gait (FOG) and receive outpatient care at the Department of Neurology, First Faculty of Medicine, Charles University and General University Hospital in Prague.

FOG will be defined for study eligibility as a score of at least 1 on MDS-UPDRS Part II, Item 2.13.

The cohort will include patients under long-term clinical follow-up. Some participants may have previously been monitored for REM sleep behaviour disorder (RBD) and subsequently developed PD.

4.2. Inclusion Criteria

All inclusion criteria must be fulfilled:

1. Diagnosis of Parkinson's disease with freezing of gait, defined as a score ≥1 on MDS-UPDRS Part II, Item 2.13.
2. A score greater than 19 on the Czech version of the Montreal Cognitive Assessment (MoCA), adjusted for years of formal education.
3. Ability to walk independently for at least 100 metres without a walking aid.

All inclusion criteria must be answered **YES** for a participant to be eligible.

4.3. Exclusion Criteria

Participants will be excluded if any of the following criteria are present:

1. Parkinson's disease dementia (PDD).
2. Other central nervous system disorders or diseases.
3. Gait impairment caused or substantially aggravated by orthopaedic or other somatic conditions.
4. Inconsistent antiparkinsonian medication dosing or changes in antiparkinsonian medication during the month preceding the assessment.

5. Severe uncorrected visual or hearing impairment that prevents reliable completion of the study assessments.

All exclusion criteria must be answered **NO** for a participant to be eligible.

4.4. Healthy Control Group

The healthy control group will consist of adults without neurological disorders or clinically significant gait impairment. Controls will be age-matched with the PD cohort and will serve as a reference group for comparison of gait and motor-cognitive performance.

The control group will undergo the same neuropsychological, standard gait, and TWT assessments as the PD group. IMU-based gait assessment will also be performed during all walking tasks. PD-specific clinical assessments and patient-reported outcome measures will be administered only to participants with PD, where applicable.

5. Recruitment and Participant Enrolment

Potential participants with PD will be identified through a review of existing clinical records at the Department of Neurology, First Faculty of Medicine, Charles University and General University Hospital in Prague.

Only patients who have previously provided consent to be contacted about research opportunities will be approached. Eligible potential participants will be contacted using their previously authorized telephone number or email address.

Individuals who express interest in participating will receive written study information describing the objectives, procedures, and requirements of the study. They will subsequently be scheduled for an initial study visit.

Before any study-related assessments are performed, participants will be given the opportunity to ask questions and will provide written informed consent.

Healthy controls will be recruited separately using [TO BE SPECIFIED] recruitment procedures.

6. Study Procedures and Assessments

All assessments will be performed during a single study visit lasting approximately 60–90 minutes, following a predefined sequence.

6.1. Baseline Clinical and Demographic Assessment

The following demographic and clinical characteristics will be recorded:

- Age and sex.
- Duration of Parkinson’s disease.
- Years of formal education.
- Relevant medical history.

- Current antiparkinsonian medication and daily dosage.
- Other medications taken regularly, including their names and dosages.

The following anthropometric parameters will also be measured:

- Body height (cm).
- Body weight (kg).
- Body mass index (BMI, kg/m²).
- Lower-limb length (cm), measured from the anterior superior iliac spine to the medial malleolus.

6.2. Clinical Assessment

Clinical assessment will include the following measures.

MDS-UPDRS Part II, Item 2.13 – Freezing of Gait

Item 2.13 will be used to assess the presence and severity of freezing of gait during daily activities. The score ranges from 0 to 4, with higher scores indicating greater FOG-related disability.

A score of at least 1 will be required for inclusion in the PD group.

MDS-UPDRS Part III – Motor Examination

The motor examination will be performed using Part III of the Movement Disorder Society–Unified Parkinson's Disease Rating Scale (MDS-UPDRS). The total score will be recorded to characterize the severity of motor impairment.

All patients with PD will undergo the motor examination in the Med-ON state.

6.3. Neuropsychological Assessment

A standardized neuropsychological battery will be administered to assess global cognitive function and specific cognitive domains relevant to motor-cognitive performance.

The battery will include:

1. **Montreal Cognitive Assessment (MoCA):** assessment of global cognitive function.
2. **Judgment of Line Orientation (JoL):** assessment of visuospatial perception.
3. **CLOX:** assessment of executive function using a clock-drawing task.
4. **Trail Making Test (TMT), Parts A and B:** assessment of processing speed, visual attention, and executive function, including cognitive flexibility.
5. **Stroop Test:** assessment of selective attention and inhibitory control.
6. **Verbal Fluency Test:** assessment of verbal fluency and executive retrieval processes.

7. **Digit Span:** assessment of attention and working memory.

The MoCA score will also be used to determine eligibility according to the predefined inclusion criterion.

6.4. Standard Gait Assessment

Walking performance will be assessed under single-task and dual-task conditions, as well as during fast walking. Inertial measurement unit (IMU) sensors will be used to record gait-related parameters during all walking conditions.

The gait assessment will be performed using a 10-metre walking Timed-Up and Go (TUG) test.

The following conditions will be performed:

- **Single-task walking:** two trials of normal walking.
- **Dual-task walking:** two trials of walking while performing serial subtraction. During the first trial, participants will subtract seven repeatedly from 100; during the second trial, they will subtract seven repeatedly from 110.
- **Fast walking:** two trials of walking at the participant's fastest safe pace.

Participants will be instructed to perform each walking task according to standardized instructions.

6.5. Trail Walking Test (TWT)

The Trail Walking Test will be used to assess motor-cognitive interference during walking involving directional changes. The TWT is a motor-cognitive dual-task paradigm adapted from the neuropsychological Trail Making Test (TMT), originally introduced as part of the Army Individual Test Battery (1944).

The test will follow the original protocol described by Klotzbier and Schott (2017). Fifteen laminated floor markers will be arranged within a 3 × 3 m testing area. An A4-sized target area (21 × 30 cm) will be placed at each designated position of the matrix.

Participants will be instructed to step on the targets according to predefined numerical or alternating numerical-alphabetical sequences.

Two TWT conditions will be administered in a fixed order.

TWT-A – Numerical Sequence

Participants will be instructed to step on the targets in ascending numerical order (1–2–3). Two recorded trials will be performed.

TWT-B – Alternating Numerical-Alphabetical Sequence

Participants will be instructed to step on the targets while alternating between ascending numbers and letters (1–A–2–B–3–C). Two recorded trials will be performed.

Before the recorded trials, participants will complete two practice trials on a practice course to familiarize themselves with the task requirements.

TWT-A will always be performed before TWT-B. The same sequence will be used for all participants.

IMU sensors will be used during both TWT conditions to record gait-related parameters. Completion time and task errors will be recorded. Errors will be documented manually by the examiner during the assessment and will also be automatically detected and evaluated by the augmented-reality (AR) software.

TWT-A and TWT-B correspond to the numerical and alternating numerical-alphabetical conditions originally described as TWT-2 and TWT-3, respectively. The revised terminology is intended to reflect their conceptual relationship to the corresponding TMT conditions.

6.6. Wearable Sensor-Based Gait Assessment

Gait-related parameters will be recorded using the APDM Opal inertial measurement unit (IMU) system.

The sensors will be used during all standard gait assessment conditions and both TWT conditions. Sensor-derived data will be used to characterize gait and mobility performance under single-task and motor-cognitive dual-task conditions.

The specific sensor configuration, anatomical placement, recorded signals, and derived gait parameters will be documented in the study technical and statistical analysis protocols.

6.7. Freezing of Gait Assessment

Freezing of gait will be assessed using the Freezing of Gait Clinical Outcome Assessment (FOG-COA) protocol.

The assessment will include the Clinician-Reported Outcome (ClinRO) component. The procedure will follow the standardized FOG-COA assessment instructions to evaluate the presence and severity of FOG.

The specific assessment tasks and scoring procedures will follow the applicable FOG-COA protocol.

6.8. Patient-Reported Outcome Measures

At the end of the assessment, participants with PD will complete the following questionnaires:

- **Parkinson's Disease Questionnaire-8 (PDQ-8):** assessment of health-related quality of life in individuals with PD.
- **Falls Efficacy Scale International (FES-I):** assessment of concerns about falling during daily activities.

These questionnaires will be administered only to participants in the PD group.

7. Assessment Sequence

The study visit will follow the sequence below:

1. Verification of eligibility and informed consent.
2. Collection of demographic, clinical, medication, and anthropometric data.
3. Clinical assessment, including MDS-UPDRS Part II, Item 2.13, and MDS-UPDRS Part III.
4. Neuropsychological assessment.
5. Standard gait assessment under normal walking, dual-task walking, and fast walking conditions, with IMU recording.
6. Trail Walking Test, including two practice trials, followed by two recorded TWT-A trials and two recorded TWT-B trials, with IMU recording.
7. Freezing of gait assessment using the FOG-COA ClinRO protocol.
8. Completion of the PDQ-8 and FES-I questionnaires by participants with PD.

Each participant will undergo the assessment protocol once, during a single study visit.

8. Data Collection and Management

Study data will include demographic and clinical characteristics, medication information, neuropsychological assessment scores, standard gait assessment results, TWT performance measures, FOG severity scores, and patient-reported outcome measures.

Wearable IMU sensors (APDM Opal) will be used during all standard gait and TWT conditions. Sensor-derived gait and mobility parameters will be recorded and processed according to the predefined study analysis protocol.

TWT performance data will include completion times and task errors. Errors will be documented manually by the examiner and automatically evaluated by the AR software.

All study data will be coded and stored in a secure research database. Direct personal identifiers will be stored separately from research data, with access restricted to authorized study personnel.

De-identified individual participant data may be made available to qualified researchers upon reasonable request, subject to applicable ethical, legal, and data protection requirements.

9. Ethical Considerations

The study will be conducted in accordance with the principles of the Declaration of Helsinki and applicable national and European regulations governing research involving human participants.

The study protocol was approved by the Ethics Committee of the General University Hospital in Prague (Etická komise Všeobecné fakultní nemocnice v Praze), approval number **101/25**.

All participants will receive written information about the study objectives, procedures, potential risks, and data handling. Written informed consent will be obtained before enrolment and before any study-related assessments are performed.

Participation will be voluntary. Participants will be informed that they may withdraw from the study at any time without consequences for their medical care.

10. Study Duration

The planned study period is December 2026 to December 2027.

Each participant will undergo a single study visit lasting approximately 60–90 minutes. No follow-up visits are planned as part of this study.

11. Data Sharing

De-identified individual participant data (IPD) and supporting study documentation will be made available to qualified researchers upon reasonable request, subject to approval by the study investigators and compliance with applicable ethical, legal, and data protection requirements.

The data intended for sharing will include neuropsychological assessment scores, wearable sensor-derived gait and mobility measures, TWT completion times and task errors, FOG severity scores, and relevant demographic and clinical characteristics.

Supporting documentation may include the study protocol, statistical analysis plan, and data dictionary.

The planned data availability period will begin in June 2028 and continue until December 2032. Requests will be reviewed by the study investigators, and approved data will be provided in a de-identified electronic format.