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Review

Gait analysis in chemotherapy-induced peripheral neurotoxicity rodent models

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ABSTRACT

Gait analysis could be used in animal models as an indicator of sensory ataxia due to chemotherapy-induced peripheral neurotoxicity (CIPN). Over the years, gait analysis in in vivo studies has evolved from simple observations carried out by a trained operator to computerised systems with machine learning that allow the quantification of any variable of interest and the establishment of algorithms for behavioural classification. However, there is not a consensus on gait analysis use in CIPN animal models; therefore, we carried out a systematic review. Of 987 potentially relevant studies, 14 were included, in which different methods were analysed (observation, footprint and CatWalk™). We presented the *state-of-the-art* of possible approaches to analyse sensory ataxia in rodent models, addressing advantages and disadvantages of different methods available. Semi-automated methods may be of interest when preventive or therapeutic strategies are evaluated, also considering their methodological simplicity and automaticity; up to now, only CatWalk™ analysis has been tested. Future studies should expect that CIPN-affected animals tend to reduce hind paw support due to pain, allodynia or loss of sensation, and an increase in swing phase could or should be observed. Few available studies documented these impairments at the last time point, and only appeared later on respect to other earlier signs of CIPN (such as altered neurophysiological findings). For that reason, gait impairment could be interpreted as late repercussions of loss of sensory.

1. Introduction

Chemotherapy-induced peripheral neurotoxicity (CIPN) is a common late toxicity of the most commonly used anticancer drugs: platinum-drugs, taxanes, vinca alkaloids, epothilones, proteasome inhibitors and thalidomide (Alberti et al., 2022). CIPN can be long-lasting or even permanent altering the quality of life of cancer survivors (Alberti et al., 2022; Briani et al., 2014). CIPN key features are related mostly to sensory disturbances affecting peripheral nerves: positive and negative signs/symptoms are present. Positive symptoms consist of abnormal sensations such as paraesthesia/dysesthesia and neuropathic pain, whereas sensory loss for the different modalities equals to the so-called negative signs/symptoms. Motor and autonomic changes are possible but usually quite limited (Alberti et al., 2022), with some variations depending on the drug administered. If large fiber sensory modality is impaired, changes in gait and balance are expected, and they are not usually related to motor alterations which can be very mild or even not present (Alberti et al., 2022; Cavaletti et al., 2019). If loss of proprioception is relevant, in fact, a condition known as sensory ataxia is developed (Cavaletti et al., 2019). Sensory ataxia due to CIPN is associated with increased patients' fear of falling (Zahiri et al., 2019) and actual increased risk of falls (Kolb et al., 2016), and with a deterioration in quality of life after chemotherapy treatment completion (Mols et al., 2014). Gait analysis could be used as an indicator of CIPN-related sensory ataxia (Chen et al., 2021), being a tool that can be implemented in in vivo studies (Bruna et al., 2020) that are warranted to pave the way to novel CIPN treatments (Cavaletti et al., 2008); in fact, so far CIPN treatment still lacks robust evidence both for pharmacological (Loprinzi

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et al., 2020) and non-pharmacological (Tamburin et al., 2022) treatment strategies (e.g., physical therapy), and, thus, preclinical studies searching for a sound biological rationale are still warranted: translational outcome measures are a key requirement to promptly transfer data from bench to bedside. Gait analysis may be useful in preclinical studies aiming at identifying compounds and/or non-pharmacological strategies to modify sensory ataxia due to CIPN (Cavaletti et al., 2008).

2. Gait analysis: an overview of possible approaches

Over the years, gait analysis in in vivo studies has evolved from simple observations carried out by a trained operator to computerised systems with machine learning that allow the quantification of any variable of interest and the establishment of algorithms for behavioural classification (Abbas and Masip Rodo, 2019) (Table 1).

A major breakthrough was the introduction of the Sciatic Functional Index (SFI), especially for the assessment of recovery improvement in the sciatic injury model (Inserra et al., 1998). This index varies its score according to the severity of damage, from 0 for nearly normal function to −100 for severe injury. It has been used mainly in unilateral peripheral nerve injuries, as the healthy contralateral side of the animal is required to apply the formula proposed by Inserra et al., 1998 (Inserra et al., 1998). However, SFI major limitation is related to the scarce number of variables that collects: paw lengths, and the paw widths, between the 1st and 5th digits and the 2nd to 4th digits. This index can be calculated in any gait analysis method, although initially, the animals' paws were dyed with a non-toxic dye and then allowed to walk on a blank sheet of paper. Technical issues might arise in analysing walking tracks, due to the development of flexion contractures, auto-mutilation, smearing of the print, dragging of the tail, or contamination with footprints.

Therefore, to obtain other gait parameters, more robust technologies were needed. Gait analysis recordings while animals walk on a transparent walkway or treadmill and subsequent analysis on the concomitant recording were then widely applied (Heinzel et al., 2020a). Two different approaches were mostly used: in one case the animal walks freely through a narrow walkway (the so called *active walk*, such as the CatWalk™ device) towards a nest-like station, whereas in the other approach, the animal is filmed while moving over a treadmill at a pre-set speed (the so called *passive walk*, such as the DigiGait™ device) (Xu et al., 2019). It is known that gait parameters could be differentially affected by the walking speed (Xu et al., 2019). Therefore, in the *active method*, in which the animal walks freely, the running speed should always be monitored and even considered in the analysis. Methods in which this speed is pre-selected may be less biased in this regard. However, it is possible that during the trial, animals (or a group of animals) may not be able to run at the same pre-selected speed as a consequence of neuropathy; therefore, this aspect should be carefully weighted too. The advantages of CatWalk™ include additional intensity data and corresponding 3D images, respect to DigiGait™, due to the Illuminated Footprints™ technique (Xu et al., 2019). CatWalk™ allows, in fact, transferring paw's pressure into fluorescence signals.

Another option in rodents is to analyse gait while they are obliged to keep walking for 2 min in the acrylic wheel (GAIT™ system) at a predetermined revolutions per minute (rpm) (Matsuda et al., 2016). Authors claim this allows animals to walk more naturally, although the hind paw would bear the dynamic weight, and could only be studied in asymmetric and hind paw models.

In addition to measuring gait in rodents, it could be of interest analysing composite functions, to evaluate distinct aspects of motor function and to determine even subtle loss of movement capacity such as walking on a ledge (Gyengesi et al., 2019) or ladder (Metz and Whishaw, 2009), eventually applying and adapting tests that are known to be used not for sensory but central ataxia and central nervous system disturbances. The Ledge test is a fair example of this; this test is mainly used in cerebellar ataxia and other neurodegenerative disease models. The examiner scores the ability of the animal to walk on the edge, using its tail as a counterbalance and balance to descend gracefully back to the cage or table using its paws. This test is scored on a scale of 0–3, with 0 representing the normal movement and 3 representing the most severe expression of ataxia. This scoring is subjective and based on the analysis of foot slips, rigidity or efficiency of the tail, or the inability to perform the test. Impaired motor function, in ataxic rodents could also be measured by the hindlimb clasping test (Chou et al., 2008; Guyenet et al., 2010; Gyengesi et al., 2019) which is performed to assess whether the mouse clasps its hindlimbs into its body or sprays its limbs when suspended by its tail. However, it should be noted that in mice the hindlimb clasping test can show a flexural response that is characteristic of a central neurological disorder (Guyenet et al., 2010).

It is possible that the movement or position of different joints (kinematics) may vary in these murine models. One form of measurement involves analysis of foot (Varejão et al., 2003) or ankle (Lee et al., 2013) placement while walking, although this may be due to contracture formation. Another analysis could be the observation of dorsal kyphosis (Guyenet et al., 2010; Gyengesi et al., 2019; Thomas et al., 2006), which could be a test to monitor ataxia and muscle strength. The experimenter positioned the mouse on a flat surface and observed the spine while the animal walked. Kyphosis could be an indicator of ataxia (Guyenet et al., 2010).

Another aspect to be considered is the robustness of CIPN animal models when devising preclinical studies to test gait analysis in rodent models. As in patients, preclinical studies assess CIPN using various variables: histopathology, neurophysiology, behavioural test (Bruna et al., 2020; Pozzi et al., 2020). It should be carefully checked if the selected model is fully reproducing CIPN features. On another note, a careful planning of experiments should be verified: gait analysis can be conditioned when nerve conduction studies are performed inserting subdermal stainless steel electrodes in paws, potentially altering animals' gait (Boehmerle et al., 2014; Monza et al., 2021a; b); therefore, nerve conduction studies are to be performed after gait analysis assessment and not the reverse. In addition, in case a relevant disease severity is induced, animals might be hesitant in moving across the walkway (Liu et al., 2018); a possible solution proposed to counterbalance this was to introduce a conditioning phase with sugar pellets (Santos, 2000). Rats were conditioned to walk through the central tunnel into two clear boxes at either end of the tunnel. Each end box has a sugar pellet reward window. The animals are placed on a food deprivation schedule before training to ensure they are receptive to a food reward. This method is repeated daily until the rat is conditioned to walk from end to end for a sugar pellet reward (Santos, 2000).

3. Gait analysis in CIPN models: literature review

For this review, only studies published up to January 24th, 2023, were considered. No restrictions were placed on year, but publications were limited by English, Spanish or Italian language. Based on the Population, Intervention, Comparator, Outcomes, and Study Designs strategy studies where gait was measured in rodents' model of CIPN were included (https://www.york.ac.uk/crd/SysRev/!SSL!/WebHelp/SysRev3.htm, accessed on 20th January 2023).

A literature search was conducted using relevant subject headings, keywords, and modifications made according to the three databases searched: Medline (Table 2), Web of Science and Scopus; modifications were made to fit each database. All articles were retrieved and exported to Rayyan (Ouzzani et al., 2016), where duplicates were removed and studies were identified and selected according to the inclusion criteria. All articles identified in the first screening process were included in the following one, in which selected articles were thoroughly read and screened for the inclusion criteria. Articles considered eligible after full-text view were included in the final analysis. Reasons for exclusion were recorded.

The following data were extracted from each article (1) general study

Table 1
Common methods to analyse gait impairment in peripheral neuropathies other than CIPN in rodent models. PNI: Peripheral nerves injuries; SFI: Sciatic Functional Index; *SFI: can be measured in any of the above methods.

Setting	Target of evaluation	Technique	Tool	Parameters	Outcomes	Pros	Cons
Active walks	Footprints	Illuminated	CatWalk™ (Heinzel et al., 2020a) Semi-Automated methods	-Print Length (distance unit): Length of the paw print -Print Width (distance unit): Width of the paw print -Print Area (distance unit): Area of the paw print -Base of Support (BoS) (distance unit): Distance between the two hind-or front paws	General parameters of gait	Possibility to simultaneously monitor motor and sensory reinnervation in various models of PNI with only one device and setup. The illumination of only those areas which are interesting for the investigator and the correlation of paw pressure and footprint intensity. Compared to DigiGait™, CatWalk™ excels at intrinsic velocity, intensity data (acquired by Illuminated Footprints™), and high-quality 3D images.	Requires meticulous training efforts to accustom the animals to the device. CatWalk™ is limited to animals of the size of rodents and ferrets Limited sensitivity to detect functional recovery after more severe types of nerve injury. It does not function in the situation that rats hardly walk. The animal could decrease velocity and compensate for gait changes that would be different at a higher speed (Piesla et al., 2009).
				-Mean Paw Print Intensity (arbitrary units): Mean intensity of the paw -Swing Phase (seconds): Swing Time of the paw -Stance Phase (seconds): Stand Time of the paw -Duty Cycle (%): Stand Time divided by Stand Time plus Swing Time	Pain-related		
				-Swing Speed (centimetres / second): Swing Speed of the paw -Regularity Index (RI) (%): Quantification of interlimb coordination	Coordination-related		
				-Run Duration (seconds) Duration of the walkway crossing	Other		
		Video techniques	Rat's head movement (Santos, 2000)	-Gait time (milliseconds)	Gait speeds	If changes in gait velocity occurred, variability could be minimised by pairing the gait-stance duration of each step with the average gait-stance duration from the 2 steps of the alternate foot immediately preceding and following it. Only a video camera and frame-by-frame playback recorders are required. In animals sustained partial toe loss, the gait-stance duration may be more reliable than any method using toe spreads (such as SFI).	Need to compare both paws, useful in asymmetric (not representative of CIPN). Dependent operator. The cost is greater than ink and paper walking tracks.
			Motorater (Altmann et al., 2016)	-Distances between the front and hind paws, diagonal distances between front and hind paws and height of the iliac crest	Locomotor ability		
				-Ratios of the basic distances of the injured versus uninjured side including "injured left hind paw to the right front paw" versus "right hind paw to left front paw" (LH-RF/RH-LF)	Coordination		
			Gait-stance duration (Walker et al., 1994)	-The time of floor contact is defined as gait-stance duration. The ratio of the injured/uninjured hind feet stance duration was calculated from paired consecutive steps when measured during steady walking	Functional recovery		
		Ink technique (Araújo-Filho et al., 2020)		-Print length (PL) -Distance between the 1st and 5th toes (TS) -Distance between the 2nd to 4th toes (IT)	Sciatic Functional Index (SFI) (Kim et al., 2007)*	Widely used metric for the pathology and potential treatment of nerve injury. The SFI is determined by comparing the geometric representation of the affected hind paw from an injured mouse or rat, and comparing it to the contralateral paw.	Need to compare both paws, useful in asymmetric (not representative of CIPN). The walking track analysis costs are relatively high, and results are frequently unreproducible because of the smearing of the footprints.
				-The print lengths of both the affected side (EPL) and the untouched side (NPL) PLF= (EPL-NPL)/NPL	Print Length Factor (PLF) (Ozkan et al., 2005)	Evaluate functional recovery.	The darkened walking alley makes it impossible to evaluate qualitative walking patterns the calculation of SFI is not easy and is time consuming. Walking speed is not identified.
				-% Functional Recovery: (%FR)= (1-PLF) x 100	Functional Recovery		
	Kinematics		Ankle Angle Measurements (Lee et al., 2013)	-Ankle angle (degrees): measured between the leg segment and the foot segment. -Ankle contracture angle	Gait kinematic information	Ankle contracture to be a useful measure of recovery (the ankle angle in toe-off phase measured during video gait analysis correlates with isometric tetanic force and as such is a useful parameter for evaluating functional recovery).	Need to compare both paws, useful in asymmetric (not representative of CIPN).

(*continued on next page*)

Table 1 (*continued*)

Setting	Target of evaluation	Technique	Tool	Parameters	Outcomes	Pros	Cons
			Toe out angle (TOA) (Varejão et al., 2003)	-TOA (the angle in degrees between the direction of progression and a reference line on the sole of the foot	Abnormal foot rotation (functional recovery after sciatic nerve injury)	Excellent correlation between SFI and TOA. Assess the foot motion in the transverse plane of walking and its underlying biomechanical consequences.	
			Kyphosis (Castillo-Mariqueo and Giménez-Llort, 2022;Guyenet et al., 2010;Gyengesi et al., 2019;Thomas et al., 2006)	-Score 0: mouse walks with a straight spine. -Score 1: a mild kyphosis but the mouse is still able to straighten its back. -Score 2: the mouse is unable to fully straighten its spine and maintained persistent mild kyphosis during locomotion. -Score 3: the mouse maintains a constant pronounced kyphosis.	Loss of muscle tone or strength in the spinal muscles.	Kyphosis is a characteristic dorsal curvature of the spine that is a common manifestation of neurodegenerative disease in mouse models. A measurement of ataxia.	Kyphosis is sensitive to an age effect.
	Tasks		Ladder rung walking tests (LRWT) (Fey et al., 2010)	-% Placements per total steps (correct, partial placement, slight slip, total slip, total miss) -Average time needed to cross the entire length of the ladder. -Digit score in each correct placement (full flexion, flexion 45°, flexion 90°)	Sensory-motor ability	The five categories of paw placement are affected differently depending on the severity of the root lesion. LRWT is a complex skilled motor tasks, which are more sensitive in detecting small impairments in limb use such as skilled paw placement. LRWT requires the integrity of all sensory-motor feedback systems, such as proprioceptive and mechanoreceptive systems.	It was also observed that forepaw placement of the contralateral (uninjured) limb was affected, though no formal assessment of this was done.
				-The spread length (in millimeters) -Ladder spread index (ratio of the spread of digits 2–4 measured in two successive video frames)	Tibial and peroneal nerve functions		
				-Ladder stance angle	Tibial nerve (plantar flexion) function		
			Ledge test (Guyenet et al., 2010; Gyengesi et al., 2019)	-Score 0: Mouse typically walks along the ledge without losing its balance, and lowers itself back into the cage gracefully, using its paws. -Score 1: mouse foot slips while walking along the ledge; the tail may present rigidity and could not be able to counterbalance well but the mouse is still able to walk and descend using its paws. -Score 2: the mouse is not effectively using its hind limbs or lands on its head rather than its paws when descending the cage. -Score 3: the mouse fells off the ledge, or nearly so, when attempting to descend back to the cage	Balance and coordination	It is a direct measure of coordination. Sensitive and easily evaluation of disease severity. A measurement of ataxia (mostly central).	Mainly used in mouse models of cerebellar ataxia and other neurodegenerative disease, including Huntington's disease and spinobulbar muscular atrophy. Ledge test is sensitive to an age effect.
			Hindlimb clasping test (Guyenet et al., 2010;Gyengesi et al., 2019)	-Score 0: If the hindlimbs are splayed outward away from the abdomen consistently -Score 1: If one of the hindlimbs is retracted toward the abdomen for more than 50% of the observation time (10 s). -Score 2: If both of the hindlimbs are partially retracted toward the abdomen for more than 50%. -Score 3: If both hindlimbs are entirely retracted for more than 50% of the time.	Motor functions	It is a marker of disease progression in a number of mouse models of neurodegeneration, including certain cerebellar ataxias. May show flexural response characteristic of lesions in the cerebellum, basal ganglia, neocortex or spinal cord pathologies. A measurement of ataxia.	

(*continued on next page*)

Table 1 (*continued*)

Setting	Target of evaluation	Technique	Tool	Parameters	Outcomes	Pros	Cons
Passive walks	Treadmill		DigiGait™ (Umansky et al., 2022; Xu et al., 2019) Semi-Automated methods	-Swing phase (manually calculated) -Duty cycle (manually calculated) -Projected area (recognized from direct recordings of the walking/running rats from the ventral direction (it was not merely the actual print of paw-floor contact)	Coordination Area data	Has advantages in fixed speed and dynamic SFI calculation. Possibility to use various software, DigiGait™, Visual Gait Lab (VGL) software.	In models that warrant neither intensity data nor SFI (e.g., sciatic nerve injury), CatWalk™ is slightly superior to DigiGait™ owing to its higher-quality images to explicitly illustrate hindpaw abnormalities. Speed pre-selected is needed for gait analysis.
	Wheel		GAIT® (Matsuda et al., 2016) Semi-Automated methods	-Swing time ratio= (swing time of the normal hind limb) / (swing time of the painful hind limb) -Number of step cycles	Abnormal step cycles (neuropathic pain)	Allow collect data of each step cycle from rats with serious injury, pain, and paralysis because of sub-spontaneous walking in the automatically rotated round wheel. Enables a natural walk based on the rodents' behavioural characteristic GAIT® system might provide a more sensitive parameter of dynamic weight bearing for evaluating chronic neuropathic pain in rats in addition to conventional tests.	It cannot differentiate types of neuronal abnormalities. Need to compare both paws, useful in asymmetric (not representative of CIPN). Used only in models of hind paws.

Table 2
Search strategy in MEDLINE database.

PICOS Components of Search Strategy and filter applied	
P	("Peripheral Nervous System Diseases/chemically induced"[Mesh] OR "Peripheral Nervous System Diseases"[Mesh terms] OR Peripheral Nervous System Disease*[tiab] OR Disease* PNS[tiab] OR Neuropath* Peripheral[tiab] OR Nerve Disease* Peripheral[tiab] OR Peripheral Nervous System Disorder*[tiab] OR "Small Fiber Neuropathy"[Mesh] OR Small Fiber Neuropathy[tiab] OR Neuropath* Small Fiber[tiab] OR "Polyneuropathies"[Mesh] OR Polyneuropath*[tiab] OR Polyneuropath* Motor[tiab] OR "Neurotoxicity Syndromes"[Mesh] OR Neurotoxicity syndrome*[tiab] OR Neurotoxin Disorder*[tiab] OR Neurotoxic disorder*[tiab] OR Neurotoxin disease*[tiab] OR Chemotherapy induced peripheral neuropath*[tiab] OR CIPN[tiab] OR Chemotherapy Induced Polyneuropath*[tiab] OR Chemotherapy induced peripheral neurotoxicit*[tiab] OR Chemotherapy Induced Neuropathic Pain [tiab] OR Platinum induced peripheral neurotoxicit*[tiab] OR Bortezomib induced peripheral neuropath*[tiab] OR BIPN[tiab] OR Taxane induced peripheral neurotoxicit*[tiab] OR TIPN[tiab] OR Cancer treatment induced neurotoxic*[tiab] OR Platinum drugs induced peripheral neurotoxicit*[tiab] OR chemotherapy induced painful peripheral neuropath*[tiab] OR Bortezomib Induced Neuropathic Pain[tiab] OR Chemotherapy induced neuropath*[tiab] OR platinum induced peripheral neuropath*[tiab] OR neuropathy induced by bortezomib[tiab] OR Bortezomib induced polyneuropath*[tiab] OR Taxane induced neurotoxic*[tiab] OR bortezomib induced neurotoxic*[tiab] OR taxane induced neuropath*[tiab] OR taxane induced peripheral neuropath*[tiab] OR bortezomib related chemoneuropathy patients[tiab] OR chemoneuropath*[tiab] OR Therapy related peripheral neuropath*[tiab] OR cancer neuropath*[tiab])
I	-
C	-
O	("Gait Analysis"[Mesh] OR Analysis Gait[tiab] OR Gait Analyses[tiab] OR "Gait/drug effects"[Mesh] OR "Gait Apraxia"[Mesh] OR Apraxia Gait[tiab] OR Apraxias Gait[tiab] OR Gait Apraxias[tiab] OR Dyspraxia of Gait[tiab] OR Gait Dyspraxia[tiab] OR Gait Dyspraxias [tiab] OR Apraxia of Gait[tiab] OR "Walking Speed"[Mesh] OR Speed Walking[tiab] OR Speeds Walking[tiab] OR Walking Speeds[tiab] OR Gait Speed[tiab] OR Gait Speeds[tiab] OR Speed Gait[tiab] OR Speeds Gait[tiab] OR Walking Pace[tiab] OR Pace Walking[tiab] OR Paces Walking[tiab] OR Walking Paces[tiab] OR "Lameness Animal"[Mesh] OR Animal Lameness[tiab] OR Animal Lamenesses [tiab] OR Lamenesses Animal[tiab] OR Gait Disorders Animal[tiab] OR Animal Gait Disorder[tiab] OR Animal Gait Disorders[tiab] OR Disorder Animal Gait[tiab] OR Disorders Animal Gait[tiab] OR Gait Disorder Animal[tiab])
S	-
Filter applied	Species: Other Animals

details: title, authors, year of publication and type of study; (2) study eligibility: model, including number of animals, age, sex, model-induced CIPN (cumulative dose, single dose and schedule), gait analysis outcomes and other outcomes to characterize the model, pros y cons of the gait analysis evaluation. The data extraction was documented in a Microsoft Excel spreadsheet.

The initial search found 987 eligible studies, 63 of which were removed during duplicate detection. Of the remaining 924 studies, 65 studies met the inclusion criteria. No paper was added from the reference list, or found with automatic alerts. A total of 14 papers were based on CIPN rodent models and were therefore assessed for the scope of this review (Fig. 1). Details of the literature search and paper selection are shown in Table 3. Considering all 12 experimental studies, a total of 140 rodents per chemotherapy group were included in the narrative synthesis (Table 3). Of the 14 paper included one was a systematic review (Heinzel et al., 2020a) and one was a methodological paper (Bruna et al., 2020), whose characteristics data are, thus, not reported in Table 3. The predominant sex tested was male (75%), and the average age was 7.68 ± 4.65 weeks in the rat studies and 8.17 ± 1.44 weeks in the mice studies. The most predominant rat strains were Dark Agouti and Sprague-Dawley (both 25%), followed by Wistar (16.66%). Four studies

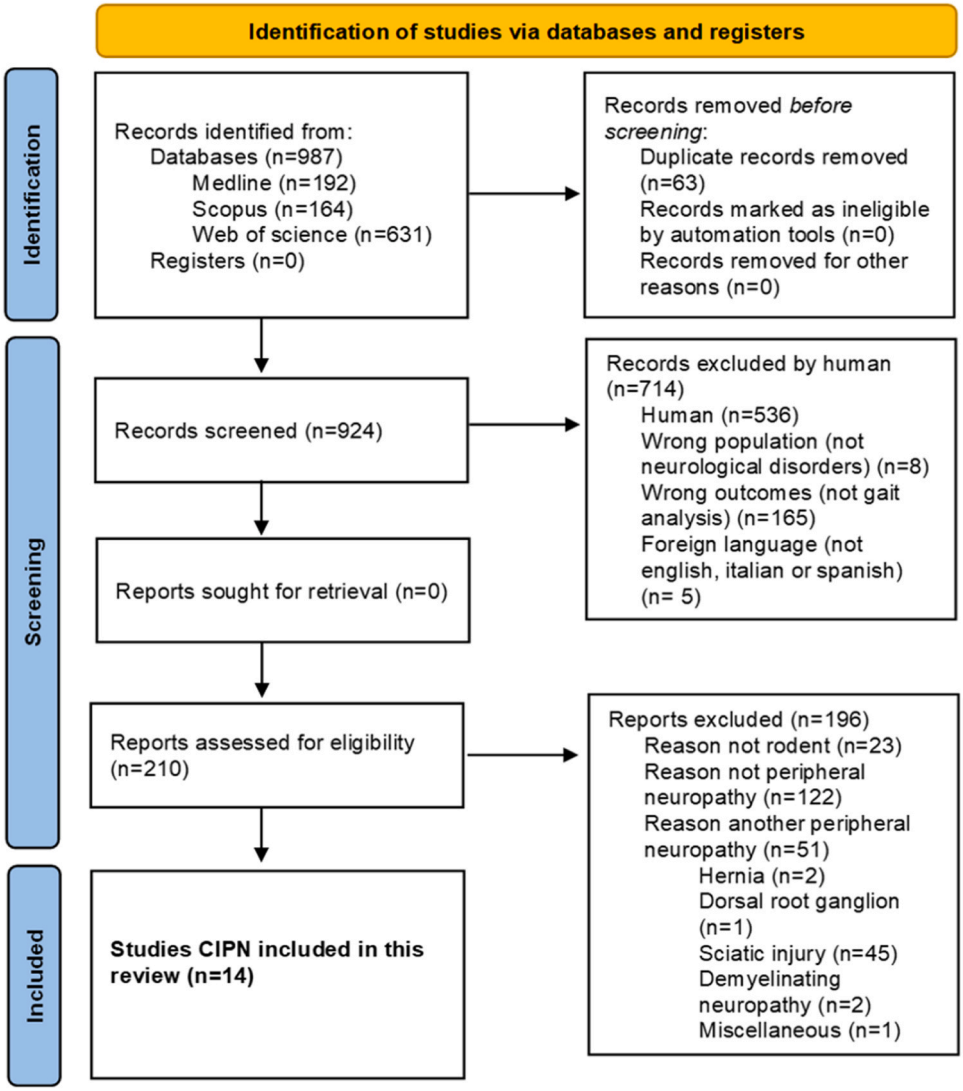

Fig. 1. Flowchart according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.

used C57BL/6 mice (33.33%) and only one CD-1 (8.33%) mice. With respect to drug-induced models, all of them were potentially neurotoxic (Velasco and Bruna, 2010). The most common model was vincristine (VCR)-induced peripheral neuropathy (6 studies, 50%), but cumulative doses varied greatly between studies, ranging from 200 μg/kg to 34 mg/kg. Five studies used cisplatin (CIS) ranging from 6 mg/kg up to 32 mg/kg and four of them use paclitaxel (PTX) with dosages ranging from 4 mg/kg up to 240 mg/kg. One study used compared carboplatin (CBDCA, 20 mg/kg) and bortezomib (BTZ, 4.8 mg/kg). Regarding the type of studies, despite the fact that all were experimental studies, only five of them randomised animals (Boehmerle et al., 2014; Huehnchen et al., 2013; Sahranavard et al., 2022; Shahid et al., 2017, 2019). In terms of the main scope, three of them aimed to characterise CIPN model with different outcomes (including gait analysis), while the others aimed at demonstrating efficacy neuroprotectant by medicating or applying the study drugs during the period of chemotherapy (secondary prevention) (Gewandter et al., 2018).

3.1. Types of gait analysis

3.1.1. Observation

Three studies carried out by the same group analysed gait via simple observation (Boyle et al., 2001; Boyle et al., 1996, 1999). Gait disturbance was assessed independently by two observers, who were blinded to the assigned treatment. The first definitive change consisted of toe-walking with an arched hind paw, which was scored as a positive result. This progressed to a general paucity of motor activity and ultimately to severe hind limb weakness, which interfered with standing and grooming behaviour. This method could be classified as quick and easy but has some disadvantages. For example, it is operator-dependent and only classifies positive and negative results once CIPN occurs; therefore, although it could classify mild CIPN or severe CIPN, it is not sensible to early onset symptoms/signs.

The results with this method can be interpreted as the number of animals developing gait disturbances in each timepoint (highlighting the percentage per group). In the first study, on day 8, only 24% of the VCR-treated animals developed gait disturbances, but by day 15 all animals were observed to be toe-walking with an arched back (Boyle et al., 1996). Subsequently, the same group tested the same approach with different CIPN models; in the CIS model, first gait impairment was observed at week 8 (15% of animals), increasing to 32% at week 7% and 100% at week 8. Onset of toe-walking gait was observed in PTX-treated rats from week 2 (16%), and by week 3 affected 100% of rats receiving PTX (Boyle et al., 1999). Finally, in the last study, only PTX resulted in gait disturbances from day 9 (17%) and 33% on day 14. The other treatments (CIS or CBDCA) did not show any alterations in gait (Boyle et al., 2001). These inconsistent findings in the different proportion of animals developing toe-walking with an arched hind-paw between two

Table 3
Characteristics of the 12 studies on gait analysis in chemotherapy-induced peripheral neurotoxicity-related sensory ataxia. #: BTZ: Affected forepaws more than hind paws; ↓: decrease; ↑: increase; Bold font means that the authors found a significant difference (time*group).

Study	Model	Model-induced Cumulative dose (single dose, schedule)	Instrument	Outcomes	Pros	Cons	Other outcomes
Patricia M. Whitaker-Azmitia et al. (1995) (Whitaker-Azmitia et al., 1995)	CIPN (Sprague-Dawley pups rats)	**Cisplatin 8 mg/kg** (1 mg/kg, twice weekly for 4 weeks)	Footprint patterns	**Degree of toeing-in** ↓ (by subtracting the interheel distance from the intertoe distance, 5 means per animals, and reported group mean)	It assesses a new marker, the convergence of the hind footprint, which could also indicate kinematics of the leg during gait.	The footprints can be erased by the mouse itself; it is necessary that the mouse is not missing any toes.	Tail-flick latency test (analgesic response) Immunocytochemistry (calcitonin gene-related peptide).
Frances M.Boyle et al. (1996) (Boyle et al., 1996)	CIPN (Dark Agouti rats)	**Vincristine 1.8 mg/kg** (0.15 mg/kg, five consecutive days the first 2 weeks, and 2 consecutive days in the 3rd week)	Observation	**Toe-walking with an arched back** General paucity of motor activity Severe hind limb weakness, which interfered with standing and grooming behaviour.	Simple and fast. It classifies on a severity scale after CIPN onset.	Dependent operator It does not detect small variations, only + or - when CIPN exists.	Tail-flick test (thermal nociceptive thresholds) Rotarod performance (in light and dark environment).
Frances M.Boyle et al. (1999) (Boyle et al., 1999)		**Cisplatin 32 mg/kg** (2 mg/kg, twice weekly until 20% weight loss occurred) **Paclitaxel 36 mg/kg** (9 mg/kg, twice weekly until 20% weight loss occurred)					
Frances M.Boyle et al. (2001) (Boyle et al., 2001)		**Paclitaxel 30 mg/kg** (5 mg/kg, for 6 consecutive days) **Cisplatin 6 mg/kg** (3 mg/kg, twice in a week) **Carboplatin 20 mg/kg** (10 mg/kg, twice in a week)					
Patricia C Contreras et al. (1997) (Contreras et al., 1997)	CIPN (CD-1 mice)	**Vincristine 34 mg/kg** (1.7 mg/kg, twice weekly for 10 weeks)	Footprint patterns	**Stride Length** ↓ **Gait support** ↓ Toe spread Internal toe spread	No specific software is needed to analyse. It is also likely that changes in gait were not the result of vincristine-induced myopathy as the changes in caudal conduction velocity are indicative of a neuropathy and there was little consistent evidence of a myopathy histologically in the soleus and gastrocnemius muscles.	It is more time-consuming to dye the animals' feet beforehand. The footprints can be erased by the mouse itself; it is necessary that the mouse is not missing any toes.	Hot-plate test (analgesic) Electrophysiological testing (caudal nerve conduction velocity) Histological analysis (percentage of degenerating fibers and total axon and myelin areas).
Petra Huehnchen et al. (2013) (Huehnchen et al., 2013)	CIPN (C57BL/6 J mice)	**Paclitaxel 240 mg/kg** (20 mg/kg, three per week for 4 weeks)	CatWalk™	**Swing phase** ↑ **Stance phase** ↓ **Duty cycle** ↓ **The print area** ↓ **of the hind paws**	Objective tool for evaluating sensory and motor neuropathy	Requires the inclusion of a non-neuropathic control group. Mice are much lighter than rats and the equipment therefore operates closer to its detection limit. Another contributing	Rotarod test (motor coordination). Von Frey Hair test (mechanical allodynia). Electrophysiological testing (caudal nerve conduction velocity and sensory nerve action potential amplitudes).

(*continued on next page*)

Table 3 (*continued*)

Study	Model	Model-induced Cumulative dose (single dose, schedule)	Instrument	Outcomes	Pros	Cons	Other outcomes
						factor is the limited sensitivity of intensity measurements: Previous studies found that at least 40% weight variation is required for a statistically significant result.	
Wolfgang Boehmerle et al. (2014) (Boehmerle et al., 2014)	CIPN (C57BL/6 J mice)	**Vincristine 200 µg/kg** (200 µg/kg, single dose) **Paclitaxel 4 mg/kg** (1 mg/kg, 4 alternative days) **Cisplatin 23 mg/kg** (2.3 mg/kg, five for 3 weeks x 2 cycles) **Bortezomib 4.8 mg/kg** (400 µg/kg, three per week for 4 weeks)	CatWalk™	**Stance phase of the hindpaws** ↓ (PTX, CIS, VIN, BTZ#) **Duty cycle** ↓ (PTX, CIS, VIN, BTZ#)	Gait analysis is not based on behaviour evoked by an artificial stimulus and is less influenced by the researcher, as well as by methodological simplicity.	Gait alterations appeared later than electrophysiological changes or mechanical allodynia.	Open fields (wellbeing of mice after injection). Rotarod test (motor coordination). Von Frey Hair test (mechanical allodynia). Electrophysiology (caudal nerve conduction velocity and sensory nerve action potential amplitudes). Histology (myelin and axon areas, changes in myelination were assessed with G-ratios which are determined by calculating the ratio of (ideal) axon diameter to (ideal) total fibre diameter).
Chang-Ning Liu et al. (2017) (Liu et al., 2018)	CIPN Wistar rats C57BL/6 J mice	**Vincristine 0.75 mg/kg** (75 µg/kg, once per day for 10 days) **1.1 mg/kg** (100 µg/kg, once per day for 11 days)	CatWalk™	Swing phase Stance phase Stride length **Print area** ↑ (only mice both front and hind paws) Print intensity	As the time needed for an animal to cross the walkway is on the order of several seconds, relatively large groups of animals can be tested in a short time span. Because the analyses are based on captured images and stored by the system, the raw data can be retrieved and quality controlled retrospectively as needed.	Footprints could be affected by weight. CatWalk™ gait analysis is more suitable for testing in mice than rats, which often showed a slower or more interrupted run on the walkway, although they were more cooperative in the pre-dose or early dosing phase.	First flick response of the tail (cold allodynia in mice). *Ex vivo* nerves mechanical testing (maximal load and the load/extension ratio). Histology (Dorsal root ganglia (DRG) and sciatic nerve).
Muhammad Shahida et al. (2017) (Shahid et al., 2017)	CIPN (Sprague-Dawley rats)	**Cisplatin 12 mg/kg** (3 mg/kg, once per week for 4 weeks)	Footprint patterns	Stride length (average distance of forward movement between each stride) Overlap between forepaw and hind paw placement (distance between the front and hind footprints on each side).	No specific software is needed to analyse	It is more time-consuming to dye the animals' feet beforehand. Only two trials were made per animal. It only reports two variables. It requires the inclusion of a control group.	Von Frey filaments (mechanical allodynia). Hot-plate test (thermal hypoalgesia). Rotarod test (motor performance).
Muhammad Shahid et al. (2019) (Shahid et al., 2019)	CIPN (Sprague Dawley rats)	**Cisplatin 12 mg/kg** (3 mg/kg, once per week for 4 weeks)	Footprint patterns	Overlap (cm between forepaw and hind paw)			Von Frey filaments (mechanical allodynia). Hot-plate test (thermal hypoalgesia). Rotarod test (motor performance).
Crystal T.Bluette et al. (2021) (Bluette et al., 2021)	CIPN (C57BL/6 J mice)	Vincristine **Nano-VCR (NP-684) 1.05 mg/kg** (0.15 mg/kg, per 7 alternatives days) **Solution-based VCR 1.05 mg/kg** (0.15 mg/kg,	CatWalk™	Swing **Stance** ↑ (VCR in front) **Cycle** ↑ (VCR in front and hind paws) Stride length Print area Print intensity	Examined the 4 paws separately but concluded that the model is symmetrical.	It is possible that other non morphological factors also play a role in animal's pain behavioural manifestation, including gait behaviours.	Histology (microscopic lesions were graded as minimal, mild, moderate, marked, or severe in L4 and L5 DRG and sciatic nerve). miRNA quantification (miR-124, miR-338, and miR-183).

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Table 3 (*continued*)

Study	Model	Model-induced Cumulative dose (single dose, schedule)	Instrument	Outcomes	Pros	Cons	Other outcomes
		per 7 alternatives days)					
S.Sahranavard et al. (2022) (Sahranavard et al., 2022)	CIPN (Wistar rats)	**Vincristine 1.4 mg/kg** (0.1 mg/kg, daily for 2 weeks)	Footprint test (hind limb was dipped in ink and they were permitted to walk on a white paper placed on the surface of the track. Analysis with ImageJ)	**Pressure of the foot** ↓ by the measurement of pixel values (VCR)	Simple and fast	Operator dependent. It reports tinted area on paper as leg pressure which can lead to bias.	Open field test (general locomotor activity). Hot-plate test (thermal hyperalgesia). Von Frey hair test (mechanical allodynia). Grip strength (muscular strength).

studies (Boyle et al., 2001; Boyle et al., 1999), could be due to the different amount of CIS cumulative dose used (32 mg/kg vs 6 mg/kg).

3.1.2. Footprint

In total, five studies measured gait using footprint patterns by dyeing the animals' paws (Contreras et al., 1997; Sahranavard et al., 2022; Shahid et al., 2017, 2019; Whitaker-Azmitia et al., 1995). Generally for analysis, all (Shahid et al., 2017, 2019) or only the hind paws(Contreras et al., 1997; Sahranavard et al., 2022; Whitaker-Azmitia et al., 1995) were dyed and the animal was placed in a lighted corridor covered with white absorbent paper. Usually at least two footprint patterns were analysed. However, the variables studied differs greatly between the studies.

In 1995, Whitaker-Azmitia et al. (Whitaker-Azmitia et al., 1995) analysed the degree of gait abnormality as the degree of toeing-in. This parameter is determined by subtracting the inter-heel distance from the inter-toe distance: a reduction of the toe-heel distance (cm) is assigned as sign of peripheral neuropathy. In this study, they showed that animals treated with CIS had a significant ($p < 0.02$) decrease in the toe-heel measure respect to the control group (1.15 ± 0.17 cm. vs. 1.61 ± 0.17 cm, respectively). However, this study used rat puppies and, therefore, gait abnormality could be due to central nervous system (CNS) toxicity: the blood-brain barrier is not intact in puppies having the same age as the ones used in this experiment, thus allowing cisplatin to reach CNS. Therefore, differ from what might be found in a CIPN exclusive model. This way of analysing gait would be more in line with kinematics or foot positioning during gait (see Table 1).

Another study (Contreras et al., 1997) measured stride length, gait support, toe spread and internal toe spread, using a similar method which represents the ability of the animal to control placement of its hind paws when walking. Administration of VCR resulted in significant reductions only in stride length and gait support of 17.3 and 10.1 mm, respectively compared to the control group (which had no change) at 10 weeks. Other works, in which all animal's paws were dyed, (Shahid et al., 2017, 2019), allowed to analyse the stride length (average distance of forward movement between each stride) on the right side and the overlap (distance between the front and hind footprints on each side) on both sides. However, they did not find any difference in CIS groups compared to the control group.

Another outcome of interest was defined as the area of footprint of the paw measured in pixels (Sahranavard et al., 2022). The group treated with VCR decrease the pressure of the foot by the measurement of pixel values in comparison with the control group (1248 pixels vs 9272 pixels); according to the Authors, this decrease would be due to a weakening of the muscles of the paws.

3.1.3. CatWalk™

Four studies used Semi-Automated methods for gait analysis (Boehmerle et al., 2014; Huehnchen et al., 2013; Liu et al., 2018; Shahid et al., 2019). This method does not rely on behaviour evoked by an artificial stimulus and is less influenced by the investigator, and it is also characterised by methodological simplicity and automaticity.

For a reliable comparison (both inter-studies or inter-groups) the gait parameters of groups it is of utmost importance that there is no difference in speed of gait among groups of animals; as already stated, in fact, the speed of gait is known to strongly affect gait parameters analysis with this technique (Deumens et al., 2007). In these four studies, the trial were regarded as successful if rodents did not stop on the runway and the cut-off time for running was 15 and 5 s for rats and mice, respectively (Liu et al., 2018; Shahid et al., 2019), or if the animal did not show a maximum speed variation greater than 60% (Boehmerle et al., 2014; Huehnchen et al., 2013) or did not exceed a walking speed of 400 mm/s (Boehmerle et al., 2014; Huehnchen et al., 2013).

As mentioned above, CatWalk™ can analyse pain-related outcomes through duration of the swing phase (no paw contact), duration of the stance phase (paw contact), and duty cycle (%) which is stand time divided by stand time plus swing time. Nevertheless, as mentioned by other Authors (Heinzel et al., 2020a) the term "pain-related" does not imply that the parameters subsumed under it are necessarily exclusively related to pain, but can for example also be influenced by impaired weight-loading due to muscle weakness or altered sensation, other than pain (e.g. numbness (Deumens et al., 2007; Li et al., 2014).

Literature data show some inconsistencies. Stance phase in hind paws were recorded as decreasing from day 10 until day 30 after the start of chemotherapy in two similar studies which used control animals to normalise the data from treated animals (Boehmerle et al., 2014; Huehnchen et al., 2013). Specifically, the reductions were by 90% after 14 days, 82% after 30 days at high doses of PTX (240 mg/kg) (Huehnchen et al., 2013), and more slightly reduced at minimal doses by 11% in PTX after 13 days, 17% in CIS after 20 days and 16% in VCR after 9 days. However, mice receiving BTZ reduced stance phase only in fore paws by 12% after 26 days (Boehmerle et al., 2014). These findings were only observed at a later time point, and thus later than the development of electrophysiological measurements and mechanical allodynia. Another study, found the opposite finding, they suggested that stance phase is increased in forepaws 10 days after VCR. It should be noted that this opposite findings, should be because of the induction of pain instead of CIPN in VCR administration in Bluette et al. study, as reported previously (Authier et al., 2003) or by the fact that 2 out of 7 mice did not show axonal degeneration in microscopy analysis (Bluette et al., 2021).

Parallel to these changes, the same findings were found in duty cycle

(as expected, because this outcome is calculated through stance phase and swing phase). Higher doses of PTX reduced duty cycle in the hind paws by 94% after 14 days, and by 92% after 30 days (Huehnchen et al., 2013). Comparing different treatments, at lower doses, Boehmerle et al. found a reduction at late points by 7% in PTX, by 12% by CIS, by 6% by VCR in hind paws. As before, BTZ induced a reduction in duty cycle only in the forepaws by 4% at the late time point (26 days after the start of chemotherapy). In contrast with these findings, a significant increase in duty cycle was documented in fore and hind paws (Bluette et al., 2021); however, a true CIPN model may not have been achieved in their study.

Another outcome reported was the print area, which is considered as a general gait parameter and it is the most reported outcome in peripheral nerve injury (Heinzel et al., 2020a). Two out of 4 studies reported significant differences in this outcome. On one hand, the first one, reported a reduction in day 30 after the start of PTX (20 mg/kg cumulative doses) by 68% in hind paws compared to baseline (Huehnchen et al., 2013). On the other hand, Lui et al. (Liu et al., 2018) reported (only in mice) an increase in print area both in fore and hind paws after 5, 12 and 15 days after first doses of VCR. This last finding should be interpreted cautiously, due to the fact that they did not find any microscopic findings in sciatic nerve or the dorsal root ganglion (Liu et al., 2018), therefore, this model might have not induced CIPN; however, sciatic nerve could be a too much proximal site to look for PTX-induced CIPN as well as DRG: it cannot be ruled out that a mild distal axonopathy was present (Pozzi et al., 2023; Wozniak et al., 2018).

4. Discussion

CIPN-related sensory ataxia is, potentially, a late neurological toxicity that impairs quality of life of cancer survivors (Cavaletti et al., 2015; Park et al., 2022; Tamburin et al., 2022). This condition is still little known and quite often confounded with a motor impairment which is not actually present (Cavaletti et al., 2019). The diminished manual dexterity and the impaired gait/balance can be impactful in patients' daily life, also decreasing their working ability, making this condition impactful both for the individual and the society (Cavaletti et al., 2023; Pike et al., 2012). Clinical trials addressing this condition, based mostly on physical treatments, are increasing in number but there is not definite evidence on the best approach for treatment and prevention of CIPN-related sensory ataxia (Park et al., 2022; Tamburin et al., 2022); this is due, mostly, to a lack of consensus on the ideal study design and outcome measures to evaluate CIPN per se in a clinical trial (Argyriou et al., 2019; Cavaletti et al., 2010), even if some indications were given in the last few years by international large study groups and experts' opinion: a combination of robust physician based scales, such as the Total Neuropathy Score (TNS®), with patients' reported outcomes is strongly encouraged (Alberti et al., 2021; Alberti et al., 2014; Cavaletti et al., 2013; Dorsey et al., 2019; Gewandter et al., 2018). However, for what regards the specific case of sensory ataxia, a formal evaluation such as gait analysis could be a relevant and powerful tool that still waits to be implemented in this setting, specifically for interventional clinical trials (Jiang et al., 2022; Wang et al., 2022). This is even truer if the preclinical setting is considered too: in absence of a defined CIPN treatment (Loprinzi et al., 2020), in fact, preclinical research is need to devise mechanisms of damage and test novel neuroprotectant drugs based on a sound biological rationale (Alberti, 2017; Bruna et al., 2020). In case of sensory ataxia, if a transition from bench to bed side is to be suggested, for sure gait analysis is a fair option to obtain a more objective evaluation in order to test novel hypotheses and then go back to the bed side. However, gait analysis in animal models can be performed with several different approaches and there is not a guideline specifically addressing its use in CIPN. Therefore, we presented a detailed description of possible strategies to analyse sensory ataxia in CIPN rodent models, addressing advantages and disadvantages of different methods available.

From the literature data presented so far, it could be suggested that semi-automated methods - which do not rely on behaviour evoked by an artificial stimulus and are less influenced by the investigator - may be of interest when preventive or therapeutic strategies are evaluated, also considering their methodological simplicity and automaticity; up to now, only CatWalk™ analysis has been tested in CIPN setting.

Notably, it should be highlighted that comparison of literature data was complicated by the fact that CIPN models differ greatly among different studies; this is a crucial issue since a different schedule and, even, a different animal strain can determine a more mild/severe CIPN, or even not inducing CIPN at all but just a nocifensive behaviour (Pozzi et al., 2020). The first indication that should be given, in fact, it is that a robust preclinical CIPN model should rely on a multimodal approach exploiting neuropathology, neurophysiology, behavioural tests, to ensure the schedule actually induced nerve damage (Bruna et al., 2020; Monza et al., 2021a; b; Pozzi et al., 2020; Pozzi et al., 2023).

Taking into account these limitations, we can state that the few available studies documented gait disturbances at the last time point, and only appeared later on respect to other earlier signs of CIPN (such as altered neurophysiological findings). For that reason, gait impairment could be interpreted as late repercussions sensory loss. Future studies should expect a decremental effect on stance phase and print area in several agents chemotherapeutic such as PTX, CIS, VCR in hind paws. We hypothesised that chemotherapy-treated rodents tend to reduce hind paw support due to pain, allodynia or loss of sensation, and an increase in swing phase could or should be observed; this theory has been documented by Huehnchen et al. (Huehnchen et al., 2013), but unfortunately did not show significant differences.

Nevertheless, alterations in gait must be interpreted with caution in these in vivo models. These changes can also be related to a possible functional adaptation to maintain an inconspicuous gait, given that rats are a prey species and try to avoid showing pain or disability to potential predators(Graham, 2016). It is, therefore, recommended to use automated gait analysis as a complementary tool(Heinzel et al., 2020b).

Some other functional tests mentioned above might be eventually explored in the future, translating some lessons learnt from central nervous system disease model, taking into account the behaviour as it is in the legde test (Guyenet et al., 2010) and LRWT (Fey et al., 2010); these tests could give an overview of coordination and motor component during gait. So far, though, in preclinical studies they were used for the measurement of cerebral ataxia or other neurodegenerative syndromes, and not for CIPN models.

5. Concluding remarks

In conclusion, it can be suggested that further studies are needed to consolidate the use of gait analysis in rodent models. It is crucial that the schedule is adequate to induce CIPN fully (e.g., not just a nocifensive behaviour) and that CIPN onset is carefully assessed with objective methods such as histopathology and neurophysiology (Alberti et al., 2020; Ballarini et al., 2022; Bruna et al., 2020; Monza et al., 2021a; b; Pozzi et al., 2023). Empowering this tool in preclinical models would be of great value since gait analysis is being introduced also in CIPN clinical trials, especially the ones exploring the role of physical therapy treatment to cure CIPN (Lopez-Garzon et al., 2022) (Park et al., 2022): having a translational outcome measure to test sensory ataxia similarly at bench and bed side would allow to promptly translate preclinical evidence to a robust clinical trial.

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Declaration of Competing Interest

None.

Data availability

No data was used for the research described in the article.

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