Intensive Functional Neurorehabilitation and Follow-up of 84 Paraplegic Dogs Affected by Intervertebral Disc Disease

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Abstract

Background

The objectives of this study were to verify whether the functionality obtained with functional neurorehabilitation intensive protocols (FNRIP) improve ambulation, promoting a new therapeutic approach, and understand the expected time for functional recovery. Furthermore, to know whether “spinal reflex” locomotion could be a functional locomotory pattern, which may improve the quality of life.

A controlled prospective clinical study using a large cohort of 84 dogs comprising mostly chondrodystrophic-breeds. The dogs were diagnosed with T10-L3 Hansen Type I, using computed tomography or magnetic resonance imaging, and treated with hemilaminectomy. All had postsurgical neurological stage 0 or 1, according to the Open Field Score (OFS), and showed either an absent or decreased flexor peripheral reflex. All patients were subjected to FNRIP within a maximum of 3 months, data were recorded on days 1,3,7,15,30,45,60,75, 90 and patients were followed-up after 8-10 days, at 1 and 6 months, and in some cases, after 1 and 2 years.

Results

Fifty-one dogs were admitted with an OFS of 1 and were discharged with an OFS of 13 (100% functionality). Of the 29 dogs that were admitted with an OFS 0, 16 were discharged (55%) in an ambulatory state, of which six dogs recovered deep pain perception (DPP) after 4 weeks, and 10 showed functional “spinal reflex” locomotion. 79.3% of these dogs achieved autonomous miction. The results were time-limited, as they were recorded within 2 to 3 months, with follow-up until 6 months. A pattern of sustained functional “spinal reflex” locomotion was observed in 30% of the dogs observed over 2 years.

Conclusions

The FNRIP are viable to regain independence and quality of life in paraplegic dogs with/without DPP, secondary to acute Intervertebral disc disease (IVDD).

1. Background

Thoracolumbar disc disease is frequently observed in chondrodystrophic dogs (Bergknut et al., 2011; Grossbard et al., 2014). The extrusion of disc material (Type I intervertebral disk disease, IVDD) causes contusion and compression of the spinal cord at different magnitudes in relation to the extrusion velocity and the mass of extruded disc material (Jeffery et al., 2013). Contusive events could lead to a chain of biochemical and vascular events that could produce myelomalacia under extreme conditions (Brisson, 2010; Muguet-Chanoit et al., 2012; Fingeroth et al., 2015; Castel et al., 2017). The compression could be reverted by removing disc material. Lesions induced by contusive and compressive events are clinically translated into clinical signs, such as paraparesis, paraplegia, and dysfunction of the reflexes and

sphincter muscles (Bergknut et al., 2013; Ingram et al., 2013). The absence of deep pain perception (DPP) has been considered a sign of poor prognosis (Olby et al., 2003; Muguet-Chanoit et al., 2012; Jeffery et al., 2016; Araújo et al., 2017; Lewis et al., 2017).

Surgery removes the extruded disc material, but the neuronal circuits persist in an interrupted manner. Functional neurorehabilitation (FNR) is a field of physical medicine and rehabilitation associated with restorative neurology. It is based on evidence of signal transmission through the lesion, both caudally and rostrally, as detected by electromyography (Tansey, 2010; Dimitrijevic, 2012; Kakulas & Kaelan, 2015). Therefore, the goal is to activate the central axon pathways traversing the lesion by synaptic stimulation (Dimitrijevic, 2012).

Based on spinal cord neuroanatomy and neurophysiology, guidelines for FNR intensive protocols (FNRIP) were developed in human medicine (Raineteau & Schwab, 2001; Gerasimenko et al., 2010; Alexeeva et al., 2011; Dietz, 2011; Gad et al., 2012; Hahm et al., 2015; García-Alías et al., 2015).

The FNRIP are intended to facilitate central nervous system (CNS) reorganization at multiple levels. It essentially provides a balance between spinal rhythm-generating circuitry plasticity and central pattern generators (CPG) (spinal locomotor network) with persistent descending pathways (Raineteau & Schwab, 2001; Dimitrijevic et al., 2015) and sensory feedback, which send signals to the CPG (van de Crommert, 1998; Rossignol et al., 2006; Barrière et al., 2008; Chen et al., 2017). Although the descending pathways are essential for producing start-stop signals, coordination, and posture (Barrière et al., 2008), short and long propriospinal interneurons can form new intraspinal circuits. Therefore, propriospinal connections can bypass the injury site and possibly mediate functional recovery (Courtine et al., 2008; Côté et al., 2017).

Thus, the FNRIPs are created through the following approaches: the locomotor training approach (van de Crommert, 1998; Edgerton et al., 2001; Wirz et al., 2005; Knikou, 2010), electrostimulation programs (Behrman & Harkema, 2000; Barbeau et al., 2002; Lavrov et al., 2006; Possover, 2014; Mehrholz et al., 2012,2017), and in cases of “possible” complete spinal cord injury (SCI) (paraplegic and DPP-negative in both hindlimbs and tail), pharmacological management (Barbeau et al., 1998; Wolpaw & Tennissen, 2001; Hayes, 2004, 2007; Fong et al., 2009; Guertin, 2013; Lewis et al., 2019).

Locomotor training can activate the spinal locomotor CPG circuitry, which interacts dynamically with afferent inputs from receptors located in the muscles, joints, and skin (Pearson, 2008; Knikou, 2010; Rossignol & Frigon, 2011). Beres-Jones & Harkema (2004) showed evidence that hip associated velocity-dependent afferent information can influence central locomotor networks.

The aim of FNR is to stimulate new intraspinal circuits and persistent residual spinal circuits through electrostimulation programs (Tansey, 2010; Edgerton & Harkema, 2011; Harkema et al., 2011; Minassian et al., 2016; Minassian & Hofstoetter, 2016). Accordingly, functional electrical stimulation (FES) leads to spinal cord plasticity and peripheral alterations with muscle fiber type conversion (Wolpaw & Tennissen, 2001; Côté et al., 2017). Furthermore, transcutaneous electrical stimulation can promote neuromodulation

effects in the spinal locomotor network of the lumbar region (Hofstoetter et al., 2013, 2014; Estes et al., 2017), and evidently plays a role in the spinal rhythm-generating circuitry (Knikou & Mummidisetty, 2014; Hofstoettart *et al*., 2017).

Reflex conditioning protocols, such as locomotor training, promote motor function restoration (Wolpaw, 2006; Chen et al., 2010; Thompson & Walpow, 2014a, 2014b, 2015). Furthermore, neuronal plasticity, owing to neurogenesis, gene activation, dendritic modifications, and changes in physiological properties, as well as glial and vascular plasticity (Wolpaw, 2010) probably to some degree also promote the restoration of motor function secondary to sodium-channel voltage activation (Wolpaw, 2007). This type of step-training increases the efficacy of synaptic connections from residual descending pathways to interneurons located near the lesion (Côté et al., 2017). Therefore, the use of multidisciplinary approaches may improve the functionality (Gomes-Osman et al., 2016; Gant et al., 2018).

To the best of our knowledge, no previous study has been conducted on FNRIP in dogs from grade 0 (open field test score, OFS 0) and 1 (OFS 1), that is, paraplegic dogs DPP negative (-) and DPP positive (+), respectively.

The primary aim of this study was to examine whether the functionality obtained with the FNRIP could improve ambulatory status with minimum coordination deficits, and thereby provide a therapeutic alternative.

The second aims were to verify the expected time for functional recovery and understand whether “spinal reflex” locomotion (SRL) could be a functional locomotor pattern that permits automaticity and a high quality of life.

2. Methods

2.1 Participants

This was a controlled prospective clinical study using a large cohort of dogs (n = 84). Most dogs were chondrodystrophic breeds, and all had T10-L3 intervertebral degenerative disc disease (Hansen Type I IVDD) diagnosed with either computed tomography or magnetic resonance imaging.

Prior surgery dogs had OFS scores of 1 (with DPP) or 0 (without DPP) (Olby et al., 2001) (Fig. 1). All OFS 0 dogs went to surgery, three to five days after DPP loss. They were all treated with hemilaminectomy and had postsurgical OFS scores of 1, with absent/decreased flexor peripheral reflexes, or 0. A total of 67 dogs were paralyzed for less than 7 days after surgery, and 17 dogs for more than 7 days after surgery. They all lacked other concomitant diseases.

Dogs OFS 0 were excluded if they presented all the criteria above but with surgical approach before 3 days or more than 5 days after injury. Also participants were excluded if they presented other SCIs, lesions outside of the T10-L3 neurolocation, higher OFS values (> 1) postoperatively, or lower OFS values

(< 1) but with a normal/increased flexor peripheral reflex, Hansen type I IVDD without any surgical procedures, and of an age greater than 7 years.

This study was conducted in dogs with IVDD, between March 2011 and January 2018 at the Arrábida Veterinary Hospital Rehabilitation Centre, after approval was granted by the Lisbon Veterinary Medicine Faculty Ethics Committee. All patients were evaluated and findings were recorded (Canon EOS Rebel T6 1300 D camera) by a Certified Canine Rehabilitation Professional (CCRP) examiner/ instructor at the University of Tennessee (AM). All the images were revised by another CCRP instructor (DM) and a non-CCRP neurologist at Lisbon University (AF).

2.2 Study design

The 84 patients enrolled in the clinical study were subjected to a neurorehabilitation consultation and were evaluated according to their history, and physical and neurorehabilitation examination (mental status, posture, gait (OFS), postural reactions, spinal reflexes, cutaneous trunci muscle reflex, palpation, and pain perception).

Regarding gait, all patients were evaluated during the same time period (3 to 7 PM), on the same 4-m sidewalk, by the same observer positioned two steps above the sidewalk. All participants were subsequently assessed in an examination room, in a controlled environment, with respect to external noise and the number of people with access. All study participants underwent an accurate evaluation regarding DPP, tested on the medial and lateral digits of the hindlimb bilaterally, on the tip and base of the tail, and on the S1-S2 dermatomes (with a 12 cm Halsted mosquito forceps). The perineal region, including the bulbocavernosus reflex, was also assessed.

All patients were examined postoperatively, and were under 7 years old, at stage 0 or stage 1, according to the OFS. They were evaluated during the initial consultation, admitted to the clinical study, and were subjected to the FNRIP within a maximum of 3 months. Either a nonsteroidal anti-inflammatory treatment (for 5 days) or corticosteroids (0.5 mg/kg *per os* SID/BID for 3–5 days) were administered to the patients. All 84 dogs that were admitted to the clinical study had either completed the recommended course of these medications or had strictly followed the neurologist´s prescription.

The patients were subjected to weekly evaluations with the same CCRP-certified examiner/instructor, and data were recorded on days 1, 3, 7, 15, 30, 45, 60, 75, and 90. At those time points, the OFS evaluations and FNRIP adaptations were recorded, along with identification of their intensity levels. Follow-ups were performed after 8–10 days, at 1 and 6 months, and in some cases, after 1 and 2 years, according to the neurorehabilitation examination criteria and OFS.

2.3. Interventions

The therapeutic approach combined the use of locomotor training, electrical stimulation, and pharmacological management within a maximum of 3 months. All patients started the FNRIP 24 h after

admission. The first day was allocated for adaptation and the patients´ first encounter with locomotor training and rehabilitation modalities.

2.3.1 Locomotor training

All dogs were acclimated to the land treadmill and began with higher body weight support (60%-80% body weight) (Dietz & Muller, 2004), which was decreased with the load tolerance (Millis & Ciuperca, 2015). The treatment sessions were supervised by a rehabilitator and commenced 2 days after admission to the clinical study.

While being supported in the harness, the patients started with quadrupedal step-training as part of their daily protocol. However, when some resistance was offered, a change to hindlimb bipedal step-training was required (Maier et al., 2009; Shah et al., 2016). During bipedal training, the forelimbs rested on a platform raised above the treadmill belt (de Leon et al., 1998) while the perineal area was stimulated, by suspending and crimping the tail or with assisted bicycle limb movements (Alluin et al., 2015).

For each training session, variables such as the walking speed and duration were increased and recorded, starting from 0.8 km/h to a maximum of 1.9 km/h (Dietz et al., 1995; Benito-Penalva et al., 2012; Meyns et al., 2014) over 5 min (4–6 times/day, 6 days/week), with the aim of reaching 20 min (2 times/day, 6 days/week) (Battistuzzo *et al*,. 2012).

Clinical study participants in quadruped training received similar stimulation with the same speed and frequency variables. The aim in this group was to reach 30–40 min (2–3 times/day, 6 days/week) (Cassilhas et al., 2016). The treadmill slope was then elevated from 10° (Maier et al., 2009) to 25° (Tillakaratne et al., 2014), to encourage forelimb-hindlimb coordination (Shah et al., 2013).

In addition, all patients began underwater treadmill training 2–7 days after admission to the clinical study. They all started at a water temperature of 26 °C (Levine et al., 2014; Sims et al., 2015) with a 5-min walk until reaching 1 h of training once a day (5 days/week), while their overtraining signs were monitored. Their speeds ranged from 1 km/h to 3.5 km/h (Engesser-Cesar et al., 2007; Leech et al., 2016).

2.3.2 Electrical stimulation

Electrical stimulation protocols were used to manage pain (interferential electrical stimulation – IES), increase muscular contraction, and possibly, increase neural connections (functional electrical stimulation – FES) and descending pathway depolarization (transcutaneous electrical spinal cord stimulation – TESCS).

2.3.2.1 Interferential electrical stimulation

This technique is a form of stimulation that has two separate channels and uses alternating currents (Sherman & Olby, 2004) through four electrodes placed on the skin near the region of spinal hyperesthesia and crossed at a 90° angle with the following parameters: acute pain, 80–150 Hz and 2–

50 ms; chronic pain, 1–10 Hz, 100–400 ms (Levine & Bockstahler, 2014; Hady & Schwarz, 2015), once a day.

2.3.2.2 Functional electrical stimulation

This neuromodulation modality that uses a short electrical pulse sequence results in spinal reflexes. Its aim is to stimulate the lower motoneuron near the motor region or through peripheral afferent stimulation (Kralj et al., 1983; Holsheimer, 1998; Hamid & Hayek, 2008). This modality was performed in all the patients with superficial electrodes, using a segmental technique. The cathode was placed on the skin region corresponding to L7-S1 and the anode was placed near the ventromedial motor region of the hindlimb flexor muscle group.

The parameters were 60 Hz, 6–24 mA (Field-Fote et al., 2005; Kapadia et al., 2014); duty cycle 1:4 ratio,,ramp up/down: 2–4 s ramp up, 8 s on time, and 1–2 s ramp down (Levine & Bockstahler, 2014). This routine was performed 2–3 times/day (5 days/week) and was discontinued, according to each patient´s neurological improvement. After the dogs were subjected to the FES modalities, they had a therapeutic window of 40 min, during which the locomotor training was conducted (Postans et al., 2004; Nooijen et al., 2009; van Hedel & Dietz, 2010).

2.3.2.3 Transcutaneous electrical spinal cord stimulation

All the patients underwent TESCS three times/day (5 days/week), which was gradually discontinued when a flexion-extension locomotor pattern appeared. The surface electrodes were placed on the paravertebral muscles (cathode at T11-T12 and anode at L7-S1, dorsal to the Iliac crest) (Rath et al., 2018; Sayenko et al., 2015, 2018; Gerasimenko et al., 2018) with a continuous current of 50 Hz, 2 mA for 10 min (Ladenbauer et al., 2010; Angeli et al., 2014; Hofstoetter et al., 2013, 2014, 2015).

2.3.3 *Pharmacological management*

During the fourth week of the described protocol, if the flexion-extension locomotor pattern was present with a DPP negative result (tested on the medial and lateral digits of the hindlimb bilaterally, on the tip and base of the tail, and on the S1-S2 dermatomes), it was added to the training protocol, and with the owner’s consent, pharmacological management. It was administered 4-aminopyridine, a K + channel-blocking compound (Hayes, 2004, 2007; Lim et al., 2014; Savin et al., 2016; Tseng et al., 2016; Zörner et al. 2016;; Lewis et al. 2019), under the following regime: 0.3 mg/kg *per os* BID for 3 days; 0.5 mg/kg BID for 3 days; 0.7 mg/kg BID for 3 days; and 1.1 mg/kg BID for 21 days.

If any side effects (seizures, diarrhoea and vomiting) occurred in any patient, they were immediately treated and withdrawn from the clinical study. The guidelines of the FNRIP in different phases are described in Table 1. Its application was consistently performed within the patient's cardiorespiratory capacity and according to the improvements observed on the functional neurorehabilitation examination and OFS assessment over a 3-month period.

Table 1
FNRIP guidelines within the 3 months hospitalization period

	Phase A (day 1–15)	**Phase B (day 15–45)**	**Phase C (day 45–90)**
LT	*5 min; 4-6x/day;* *0.8 km/h – 1.2 km/h.*	*10 min; 3x/day;* *1.2–1.5 km/h;* *With or without inclination: 5º-10º.*	*20–40 min; 2x/day;* *1.5–1.9 km/h;* *With or without inclination: 5º-10º.*
UWTM	*5–10 min; 1x/day;* *1–2 km/h.*	*10–30 min; 1x/day;* *2–2.5 km/h.*	*30–60 min; 1x/day;* *2.5– 3.5 km/h.*
ES	• *IES (1x/day)* • *FES (3x/day)* • *TESCS (3x/day)*	• Without IES • *FES (2x/day)* • *TESCS (2x/day)*	• Without IES • *FES (1-2x/day)* • Without TESCS
Pharmacological management	-	*4-AMP*	*4-AMP*
Time spent	*90 min/day* *(+/- 30 min)*	*120 min/day* *(+/- 30 min)*	*150 min/day* *(+/- 30 min)*

Abbreviations: FNRIP, Functional Neurorehabilitation Intensive Protocol; LT, Land Treadmill; UWTM, Underwater Treadmill; ES, Electrostimulation; IES, Interferential electrostimulation; FES, Functional electrostimulation; TESCS, Transcutaneous electrical spinal cord stimulation

2.3.4 *Supportive care*

The patients in the clinical study had neurogenic bladders. Thus, their bladders were expressed manually 3–4 times/day (Martins & Ferreira, 2018). The urine was monitored daily for odor and color changes. If there was a suspected urinary tract infection, urine culture (cystocentesis) and specific antibiotic treatment were administered.

The dogs were maintained under a full-time hospitalization regime. They were able to rest on soft beds with multiple disposable absorbent pads and encouraged to maintain sternal recumbency. Dogs were fed three times per day with an intake increase of 30% and hydric support of 100–120 ml/kg was orally administered after resistance training alternated with strength training, according to the patient's needs. At the end of the day, class IV laser therapy was administered to reduce pain at trigger points (Bennaim et al., 2017).

All dogs were trained during the day, starting at 9:00 a.m. and finishing at 7:00 p.m. They were assisted only by veterinarians and veterinary nurses, who had taken the CCRP course.

2.4. Outcome measures

All patients were assessed by neurological examination every 5–7 days by the same certified CCRP examiner/instructor. The measured outcomes, including the OFS values, were evaluated at baseline (day of admission) and on days 3, 7, 15, 30, 45, 60, 75, and 90 after FNRIP implementation. The presence of DPP, the flexor reflex, flexion-extension locomotor pattern, and postural standing were investigated. This facilitated the establishment of an accurate and systematic evaluation of functionality among patients. Patients were considered functional if they showed functional “spinal reflex” locomotion (FSRL), and OFS of 13/14), and were discharged.

We defined functionality as the patient’s ability to stand up, maintain postural standing, take at least three steps, and engage in voluntary or automatic micturition and defecation. Based on neural reorganization, FSRL can be defined as an “involuntary” movement with the autonomous ability to stand, take steps, and engage in voluntary or automatic micturition and defecation, thereby giving the patient independence and autonomy. Autonomous ability in movement control suggests that parts of the brain and spinal cord can probably activate movements with some conscious control (Shik & Orlosky, 1976).

However, with nonfunctional “spinal reflex” locomotion (NSRL), although the patient demonstrates the presence of a flexion-extension locomotor pattern, they do not have the ability to stand or promote the step-cycle.

After the end of the study, dogs that become DPP +, or DPP - but with FSRL or NSRL, were discharged and released to owner´s guardianship. In case of progressive myelomalacia (ascending/descending), or in DPP - dogs that don´t recover, and upon owner’s request, euthanasia was considered.

The method of euthanasia was performed using induction with Propofol through intravenous (IV) administration (cephalic vein), followed by Pentobarbital IV administration after the dog fall asleep, within a quiet room.

2.5 Statistical analysis

It is possible to assume normality of the data with an appropriately large sample size (> 40) (Ghasemi & Zahediasl, 2012). The quantitative, qualitative, and categorical data were analyzed using the IBM SPSS Statistics software, Version 22 (International Business Machines Corporation), and the results were interpreted at a level of significance of $p \leq 0.05$. The categorical data were presented as frequencies and proportions (95% confidence interval).

The clinical study was designed to investigate the outcomes and the changes in the OFS. The means and medians were calculated for the OFS at each time point. Thus, all 84 dogs were grouped and analysed according to the time of their discharge. Chi-squared tests and *t*-tests were used for inferential statistics regarding variables with a normal distribution.

Results

All 84 dogs met the inclusion criteria, and the results of their evaluations were recorded after FNRIP implementation. Most dogs were of chondrodystrophic breeds. The French Bulldog was the most common (26 dogs), followed by a high variability of breeds, with 17 Dachshunds; nine Yorkshire terriers; seven Pekingese; six Poodles; five small-legged, long-bodied mixed breeds; four Beagles; and two each of Portuguese Podengos, Jack Russell Terriers; Pinschers, and Cocker Spaniels; and one Basset Hound and Chihuahua each. There were 31 females and 53 male participants with no significant differences in age ($p = 0.756$) or weight ($p = 0.453$). The mean age was 4.15 years and the mean weight was 9.131 kg (Table 2). The most frequently affected neurolocation was T11-T12 in 27.4% (23/84) of the subjects (Fig. 2). At the time of presentation, all dogs were admitted with an OFS of 0 or 1, and 79.8% (67/84) had been paralyzed for less than 7 days and 20.2% (17/84) for more than 7 days.

Table 2
Population characterization ate admission (n = 84)

Variable	Mean (SE)	95% CI
Age (years)	4.155 (0.1538)	3.849–4.461
Body weight (kg)	9.131 (0.5038)	8.129–10.133
Abbreviations: CI, confidence interval; SE, standard error		

Fifty-one dogs, or 60.8% (51/84) of the participants, were admitted with an OFS of 1 and were discharged when they achieved an OFS of 13. A total of the 33 dogs, or 39.2% of the participants were admitted with an OFS of 0 (33/84), among which, 16 were discharged in an ambulatory state: Ten dogs had an OFS of 0 – FSRL, and six dogs had an OFS of 13 with recovered DPP after 4 weeks. In dogs that were DPP +, the OFS facilitated evaluation at each time point and throughout the progressive stages essential for achieving functionality. The details are presented in the flow diagram in Fig. 3, as well as the representative evaluation graphics in Fig. 4.

According to the OFS, no improvements were observed from days 1 to 3 in any of the 84 dogs. After one week of FNRIP implementation, all 51 dogs admitted with an OFS 1 had a classification ≤ 9. By day 15, all dogs had completed FNRIP phase A. Twenty dogs that were admitted with an OFS of 1, less than 7 days post-injury, were medically discharged with an OFS of 13. They showed only mild residual proprioceptive deficits, with clear improvements (Fig. 4A).

Among those 20 dogs, during an 8-10-day follow-up consultation, 100% showed no sensory decline. However, only 60% (12/20) presented at the next follow-ups (at 1 month and 6 months) with improved proprioceptive deficits, as manifested by a 1-point increase in their OFS (14). Three dogs presented at the first two follow-ups and showed an improved OFS of up to 14. Five dogs maintained an OFS of 13, although four of those missed the last follow-up consultations.

On day 30, 21 dogs that were admitted with an OFS of 1 received a medical discharge with an OFS of 13 (Fig. 4B). Furthermore, 28.6% (6/21) of that number showed improvements in their OFS (from 13 to 14) at

the first follow-up. Three dogs missed the 1-month follow-up and eight dogs missed the 6-month follow-up The remaining 33.3% (7/21) showed a one-point increase in their OFS.

Five dogs that remained at FNRIP phase C showed improvements in their OFS at the time of medical discharge, with an OFS of 13 on day 60 (Fig. 4C). Follow-up on these dogs indicated improvements at the first follow-up in three dogs. Only one dog missed the last follow-up. On day 90, five dogs were discharged with an OFS of 13, all of which were re-evaluated during the three follow-ups. Four of those five dogs showed an increase in the OFS (14).

All dogs that were admitted with an OFS 0 (n = 33) showed no change in the OFS until day 15. Four dogs left the study within the 15-day period, (represented by the soft blue line in Fig. 4A), one euthanized and 3 presented descending myelomalacia. However, the 29 dogs that remained showed the presence of a flexion-extension locomotor pattern until day 30.

At the same time point (day 30), two dogs recovered DPP, and were medically discharged on day 60 with on OFS of 13 (Fig. 4C). Three dogs recovered DPP on day 45, and one on day 60. Each of those dogs was discharged on day 90 with an OFS of 13, after 4-amynopiridine administration (Fig. 4D). Thus, a total of 21% (6/29) dogs recovered DPP.

On day 60, four dogs had medical discharge with an OFS of 0 – FSRL, three dogs with an OFS of 0 – NSRL and two dogs with an OFS of 13, as mentioned before (Fig. 3). On day 90, six dogs were discharged with an OFS of 0 – FSRL, 10 dogs with an OFS of 0 – NSRL, and four dogs with an OFS of 13 (Fig. 3).

All four dogs with FSRL that were discharged on day 60 were re-evaluated at the 8-10-day and 30-day follow-ups, and they maintained autonomous functionality. In addition, the six dogs that were discharged with FSRL on day 90 attended the three follow-ups and maintained an ambulatory state with minimal coordination deficits.

Outcome measures regarding the OFS mean and median scores show no obvious increase in continuous scores at each time point, given that some dogs remained in treatment and others were discharged on days 15, 30, 60, and 70 (Table 3). However, regarding the transition time points during treatment (days 7, 45 and 75), an increase was observed at the maximum range classification, with OFS scores of 9, 11, and 12, respectively. The OFS means at day 15 (5.79) and day 30 (5.95) were higher than those at day 60 (4.52) and day 90 (4.64) (Table 3).

Table 3
OFS score measures at each time point

Time points	Mean (SE)	95% CI	Median (range)	95% CI
Day 1	0.62 (0.053)	0.51–0.71	1.00 (0–1)	1.00–1.00
Day 3	0.62 (0.053)	0.51–0.73	1.00 (0–1)	1.00–1.00
Day 7	3.24 (0.360)	2.54–3.96	3.50 (0–9)	1.00–4.00
Day 15	5.79 (0.576)	4.61–6.96	7.00 (0–13)	3.00–8.00
Day 30	5.95 (0.747)	4.54–7.29	5.00 (0–13)	0.00–9.00
Day 45	3.55 (0.738)	2.19–4.88	0.00 (0–11)	0.00–5.00
Day 60	4.52 (0.883)	2.76–6.24	0.00 (0–13)	0.00–9.00
Day 75	4.04 (1.047)	2.07–6.14	0.00 (0–12)	0.00–10.00
Day 90	4.64 (1.199)	2.32–6.96	0.00 (0–13)	0.00–13.00
Abbreviations: CI, confidence interval; SE, standard error				

Chi-squared tests revealed a statistically significant relationship between a DPP positive test result at admission and the ability to achieve functionality (p = 0.000). The same type of relationship (p = 0.000) was observed between DPP positivity and the time to discharge. Very few dogs admitted with an OFS 1 required up to 3 months before discharge (0.1%).

No significant relationships were observed among the weight and age categories, and the ability to reach an ambulatory state, p = 0.219 and p = 0.844 for the Chi-squared tests and *t*-tests, respectively. Furthermore, no significant relationships were observed in the time post-injury to reach functionality (p = 0.861).

Total functionality was considered in dogs that recovered to an OFS of 13 (n = 57) and an OFS of 0 – FSRL (n = 10), which was observed in 79.8% of the patients (67/84). In the sub-group of dogs admitted with an OFS of 0 that remained DPP - until the fourth week after admission, the functionality was 55% (16/29). Six dogs recovered DPP, and 10 dogs achieved and maintained an OFS of 0-FSRL. Regarding urinary ability, 79.3% (23/29) achieved autonomous miction.

Discussion

Evidence of FNRIP efficacy has been reported by various studies (Côté et al., 2018). Such findings indicate that multidisciplinary protocols can enable neural reorganization associated with synaptogenesis, and support the introduction of locomotor training as a primary rehabilitation modality (Lavrov et al., 2006; Martins, 2015; Mehrholz et al., 2012, 2017). During the present study, 67 dogs regained functionality and 57 achieved an OFS of 13, with only conscious/sub-conscious proprioceptive

deficits. Ten dogs achieved functional “spinal reflex” locomotion (OFS 0 – FSRL), contributing to an overall result of 79.8% (67/84) functionality.

The study population was characterized by a mean age of 4.15 years and a mean weight of 9.13 kg, similar to the characteristics reported by Aikawa et al. (2012), Gallucci et al. (2017), and Zidan *et al*. (2018). The most frequently noted breed was the French Bulldog, which was not consistent with the report of Ruddle et al. (2006) or Zidan *et al*. (2018), who reported the highest frequency in Dachshunds. The lesion site in the present study was similar to that of most other studies for anatomical reasons (Jeffery et al., 2016; Gallucci et al., 2017; Zidan *et al*., 2018).

Neurological progression was not observed between days 1 and 3, and dogs maintained either an OFS of 0 or 1. This indicated a pre-selected population of 51 dogs with an OFS of 1, with absent/decreased flexor peripheral reflexes (inclusion criteria) and dogs with an OFS of 0. All dogs were referred by veterinary neurologists, because of exuberant epidural haemorrhage with venous sinus involvement that could have compromised their functional recovery (Amsellem et al., 2003).

Twenty-nine dogs with an OFS of 0 showed no recovery of DPP until the fourth week. Two recovered on day 30 after FNRIP implementation (Fig. 4C) and the remaining four recovered following the administration of 4-aminopyridine (Fig. 4D). Therefore, 21% (6/29) recovered owing to the synaptic and anatomical neuroplasticity achieved by the possible combination of locomotor training, electrostimulation, and in some cases, 4-aminopyridine, which unlocked a possible lack of connection between the brain stem and spinal locomotor network silenced by inhibitory effects (Mehrholz et al., 2012, 2017).

In the present study, an overall functionality of 55% (16/29) was observed in dogs with an OFS of 0, 21% (6/29) of the dogs recovered DPP at a later time point (after day 30), and 35% (10/29) achieved FSRL, within a maximum of 3 months. Recovery of DPP after 30 days is reflective of the pre-selected population, and is suggestive of “complete” spinal cord injury, or unsuccessful neurological cases.

According to Jeffery et al. (2016), DPP recovery is subjective. Thus, it is essential to restrict the evaluation at each time point and, in order to decrease subjectivity, to ensure that it is assessed by the same assessor, at the same time of the day and in the same environment, as performed in the present study. Also, it is important to mention that the authors believe that in the cases of FSRL (10/29), and given the coordination in the locomotor pattern achieved, there was a possible evidence of residual descending pathways, in agreement with Lewis et al. (2018).

The results of the present study are time-limited. The FSRL was achieved within 2–3 months and all dogs were closely monitored at each time point (days 7, 15, 30, 45, 69, 75, and 90). In addition, the follow-ups over 6 months showed a sustained FSRL in 30% of the dogs observed over 2 years without locomotory differences. There are no other studies with such prolonged follow-ups, which proves that this type of locomotion is sustainable over time and can be achieved in 2–3 months with intensive neurorehabilitation protocols.

For Jeffery et al. (2016), this type of locomotion could be developed usually within many months, and for Aikawa et al. (2012) was achieved within approximately 9 months. For Olby et al. (2003), 38.8% could achieve this locomotion within a mean of 37.6 weeks.

Regarding dogs with an OFS of 1, reported success rates after surgical approach can range from 85–98% (Olby et al., 2003, 2004; Loughin et al., 2005; Ruddle et al., 2006; Aikawa et al., 2012; Draper et al., 2012; Ingram et al., 2013; Langerhuus & Miles, 2017). However, comparison between studies can be difficult given the definition of success. Most studies reported success as achieving ambulatory state, without considering proprioceptive ataxia (Hodgson et al., 2017). Contrary to other reports, in the present study 80% of dogs had medical discharge within 30 days of FNRIP implementation, but with an OFS of 13 and minimum neurological deficits

In the present study there was a total functionality of 79.8% (67/84). Complete recovery could be attributed to the application of bipedal/quadrupedal locomotor training. This form of training is a rehabilitative procedure that promotes repetitive and progressive practice of the flexion/extension locomotor pattern (Harkema et al., 2012; Escalona et al., 2017). It can activate afferent inputs Ia and Ib due to muscle spindle stretching. This may have a direct connection with the hip mechanoreceptor joint (Bouyer & Rossignol, 2003; Dietz & Muller, 2004; Pearson, 2008; Rossignol & Frigon, 2011) and can activate the spinal locomotor network and CPG, facilitating recovery of the flexion/extension locomotor pattern (Thompson et al., 2013; Thompson & Wolpaw, 2014a; Solopova et al., 2015). In addition, sensory cutaneous receptor stimulation can be achieved by contact pressure on the treadmill (land and underwater) (van de Crommert et al. 1998; Guertin 2014), allowing the regulation of swing and stance locomotion phases (Dietz, 2011).

One of the possible explanations lies in the association between locomotor training and FES that may have facilitated a possible conversion of type II fibers to type I (Côté et al., 2017). This is essential for postural support while standing (Postans et al., 2004). Depending on the cathode-anode orientation, it can also lead to the potential regeneration and activation of new connections (Thompson & Wolpaw, 2015).

In addition, the authors believe that TESCS could promote the stimulation of residual motor descending pathways, and therefore, the spinal locomotor network (Minassian et al., 2016; Hofstoetter et al., 2014, 2015).

The results obtained in the present study were neither age nor weight-dependent, which was inconsistent with most previous studies (Olby et al., 2003; Ruddle et al., 2006; Gallucci et al., 2017). Also, there was no possible comparation between this study and Zidan *et al*. (2018), given the differences among population groups, with 51 paraplegics with absent/decreased flexor reflex and 33 dogs with an OFS of 0, in contrast to 9 non-ambulatory paraparetic dogs and 6 paraplegic dogs from the other study.

Considering the functional outcome, this study found close results compared to those presented by Langerhuus & Miles (2017) ´ systematic review and meta-analysis, but they were completed within a time

frame of 2–3 months.

No cases of self-mutilation were observed, as there was an absence of paraesthesia through the FNRIP that allowed neuromodulation (Smania et al., 2010). In contrast to the reports of other authors (Olby et al., 2003; Aikawa et al., 2012), no urinary tract infections were observed. This could be attributed to the full-time hospitalization regime with higher levels of supportive care, electrical stimulation protocols (Ladouceur & Barbeau, 2000; Sims et al., 2015), and greater anatomical neuroplasticity with neural reorganization (Wolpaw & Tenissen, 2001). Thus 79.3%, all OFS 0-FSRL and OFS 0-NSRL patients had medical release with autonomous miction.

There were several clinical study limitations. One was the experimental design without inter-observer and intra-observer examination. Nevertheless, images were reviewed by independent two observers (neurologist and CCRP). Additionally, no scale was applied to dogs without DPP. Although a specific scale exists, it could not have been included in the present study because it was not used consistently for all cases (Martins et al., 2018). Furthermore, it is important to note the need for future studies that would more accurately specify various scores that are applicable to dogs without DPP (OFS of 0), which could be related to their possible functionality.

Conclusions

The present clinical study demonstrated that intensive, multidisciplinary, functional neurorehabilitation protocols, including locomotor training and electrostimulation programs, applied to a pre-selected group of patients with an OFS of 1, may result in functional clinical recovery (100%). However, these methods were unable to reduce the time of recovery. In addition, for patients with an OFS of 0, the association of these protocols with the administration of 4-aminopyridine led to a 55% recovery rate over a period of up to 3 months. These findings may facilitate our determination of the answer to dog owners´ most frequent questions about the time that is necessary to achieve functionality and urinary ability in cases of a lack of neurological success.

The FNRIP showed the possibility of helping dogs with functional “spinal reflex locomotion”. Subjects demonstrated quadruped locomotion, balance, and autonomous micturition in higher numbers and within a shorter time frame. This indicated possible motor recovery, based on the level of independence and animal welfare. We concluded, therefore, that FNRIP is an asset in the discipline of restorative neurology in veterinary medicine.

Abbreviations

CCRP - Certified Canine Rehabilitation Professional

CNS - Central Nervous System

CPG - Central Pattern Generators

DPP - Deep Pain Perception

FES - Functional Electrical Stimulation

FNR - Functional Neurorehabilitation

FNRIP - Functional Neurorehabilitation Intensive Protocols

FSRL - Functional “Spinal Reflex” Locomotion

IES - Interferential Electrical Stimulation

IV - Intravenous

IVDD - Intervertebral Disc Disease

NSRL - Nonfunctional Spinal Reflex Locomotion

OFS - Open Field Score

SCI - Spinal Cord Injury (SCI)

SRL - Spinal Reflex Locomotion

TESCS - Transcutaneous Electrical Spinal Cord Stimulation

Declarations

Ethics approval and consent to participate

Study project (N/Refª 001/2019) approved by the ethics committee of CEBEA (Committee for Ethics and Animal Welfare) at the premises of the Faculty of Veterinary Medicine (University of Lisbon) on January 3, 2019. Certificate of approval is presented above.

All owners were informed and signed a consent form before patients’ admission.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

AM was the Certified Canine Rehabilitation Professional (CCRP) examiner/ instructor at the University of Tennessee that performed all neurorehabilitation examinations and re-evaluations, and also the major contributor in writing the manuscript. DG and AC were members of the neurorehabilitation team that helped in the protocol execution. IV performed the statistical analysis. AM and AF designed the study protocol. DM and AF revised all images recorded regarding the neurorehabilitation examinations. All authors read and approved the final manuscript.

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Figures

Stage 1	
0	No pelvic limb movement and no deep pain sensation
1	No pelvic limb movement with deep pain sensation
2	No pelvic limb movement but voluntary tail movement
Stage 2	
3	Minimal non-weight-bearing protraction of the pelvic limb (movement of 1 joint)
4	Non-weight-bearing protraction of the pelvic limb with >1 joint involved <50% of the time
5	Non-weight-bearing protraction of the pelvic limb with >1 joint involved >50% of the time
Stage 3	
6	Weight-bearing protraction of pelvic limb <10% of the time
7	Weight-bearing protraction of pelvic limb 10% to 50% of the time
8	Weight-bearing protraction of pelvic limb >50% of the time
Stage 4	
9	Weight-bearing protraction 100% of the time with reduced strength of pelvic limb. Mistakes >90% of the time (eg, crossing of pelvic limbs, scuffing foot on protraction, standing on dorsum of foot, falling)
10	Weight-bearing protraction of pelvic limb 100% of the time with reduced strength. Mistakes 50 to 90% of the time
11	Weight-bearing protraction of pelvic limb 100% of the time with reduced strength. Mistakes <50% of the time
Stage 5	
12	Ataxic pelvic limb gait with normal strength, but mistakes > 50% of the time (eg, lack of coordination with thoracic limb, crossing of pelvic limbs, skipping steps, bunny-hopping, scuffing foot on protraction)
13	Ataxic pelvic limb gait with normal strength, but mistakes made < 50 % of the time
14	Normal pelvic limb gait

Figure 1

Open Field Score (OFS) - The 5 stages of recovery of use of pelvic limbs in dogs with spinal cord injuries (Olby et al. 2001).

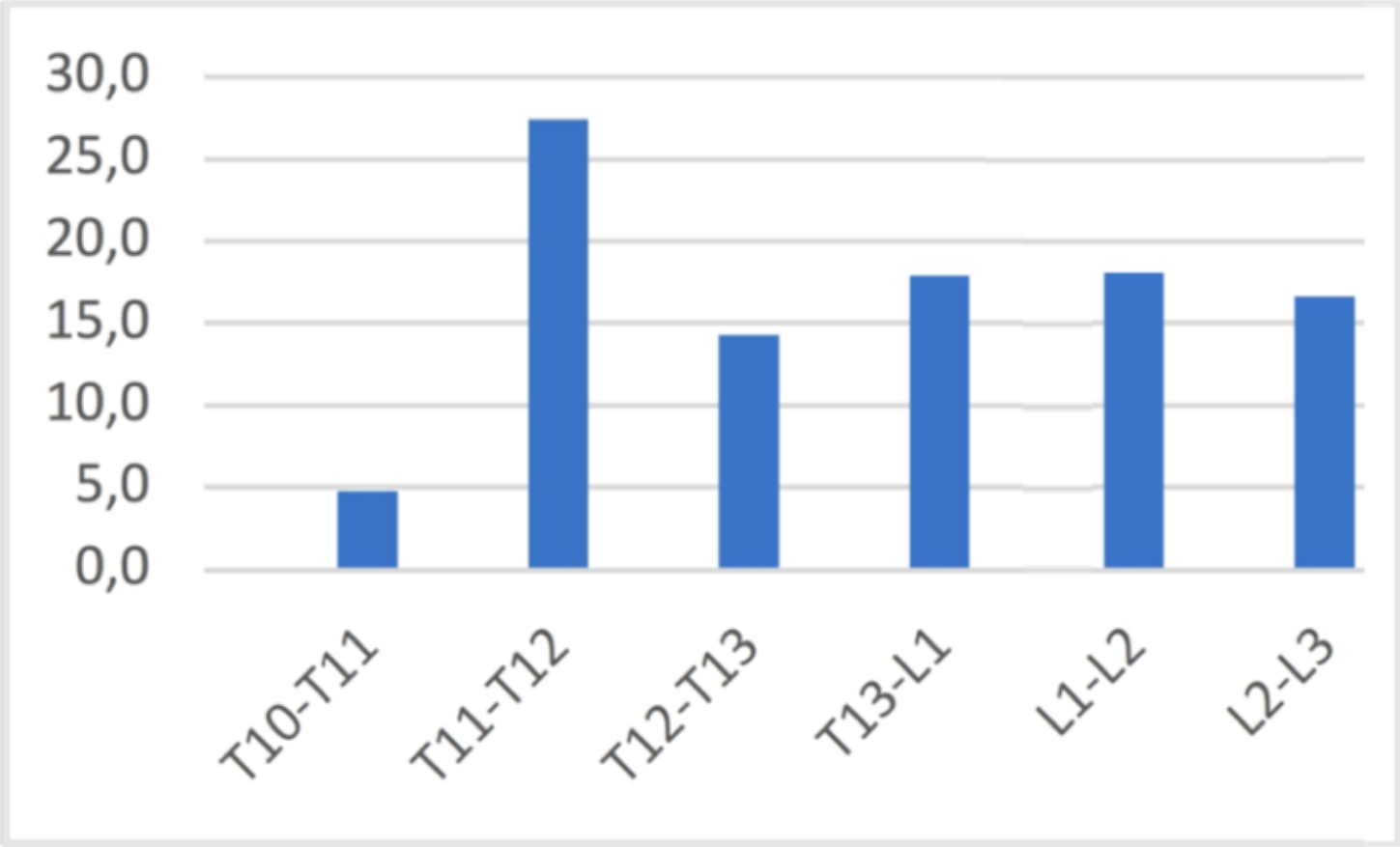

Figure 2

Neurolocation site of spinal cord injury in 84 dogs. Abbreviations: T, thoracic; L, lumbar

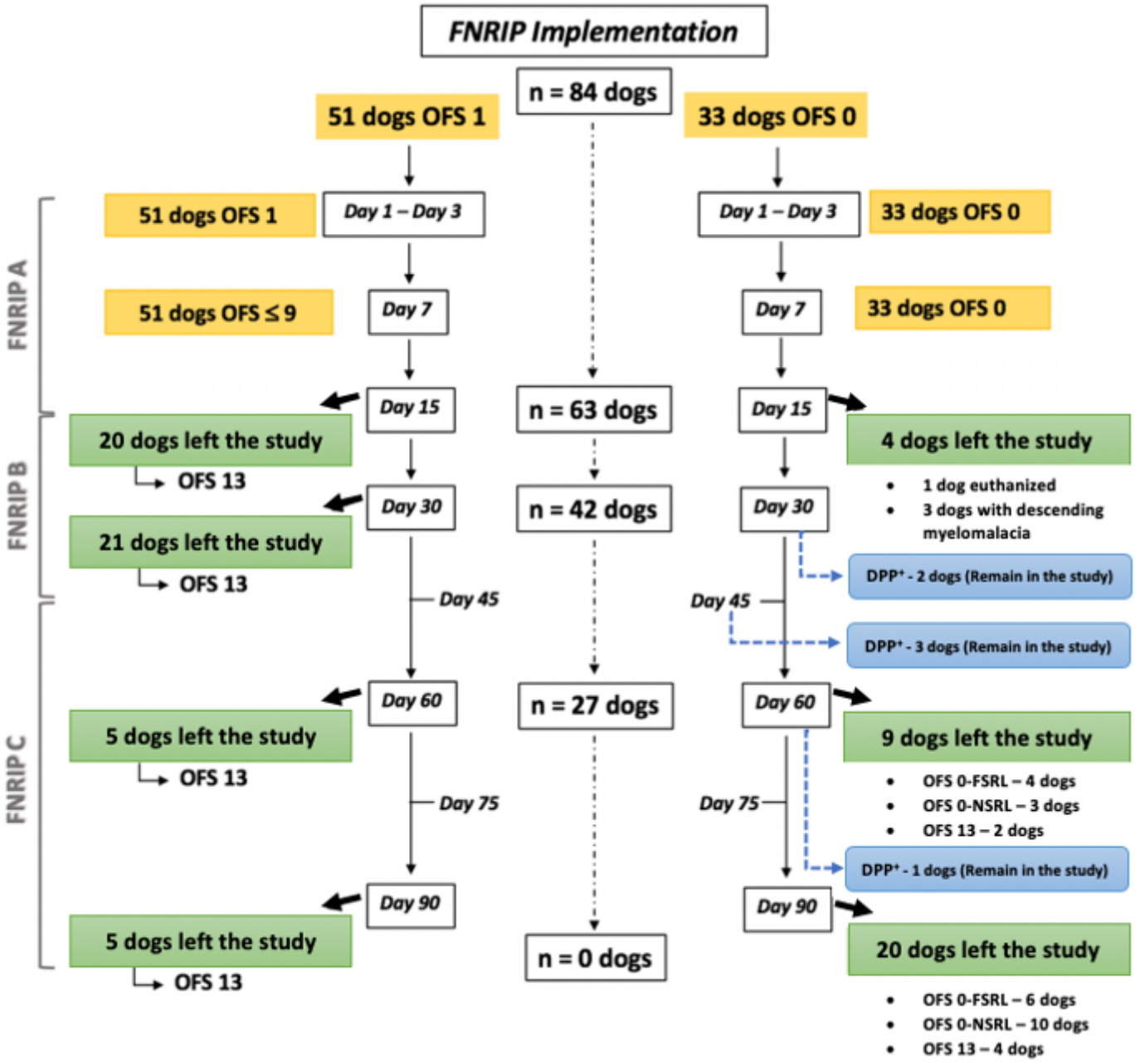

Figure 3

Flow diagram (as recommended by Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines) illustrating the dogs evolution in the prospective clinical study, demonstrating the progressive stages during the days after FNRIP implementation. Abbreviation: OFS – Open Field Scale (Olby et al. 2001); FNRIP – Functional Neurorehabilitation Intensive Protocol; FNRIP A – Protocol phase A; FNRIP B – Protocol phase B; FNRIP C – Protocol phase C; FSRL – Functional “spinal reflex”locomotion; NSRL – Non-functional “spinal reflex” locomotion; DPP + - Deep pain perception recovery

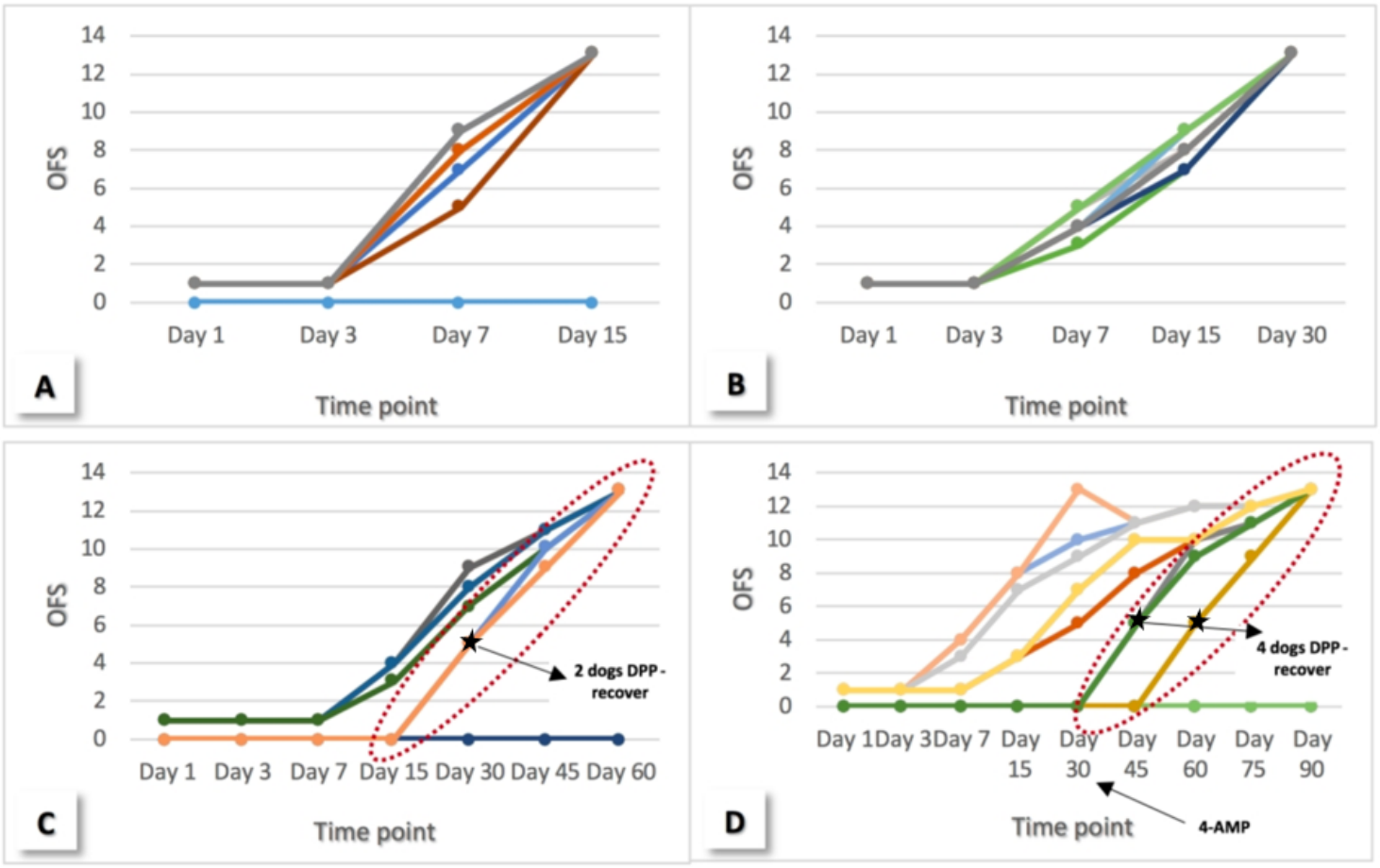

Figure 4

Recovery curves regarding OFS scores A - OFS evaluation regarding the sub-group (n=24) that had medical discharge within 15 days after FNRIP implementation. (Soft blue line: 1 dog euthanized; 3 dogs with descending myelomalacia) B - OFS evaluation regarding the sub-group (n=21) that had medical discharge within 30 days after FNRIP implementation. C - OFS evaluation regarding the sub-group (n=14) that had medical discharge within 60 days after FNRIP implementation. – Deep pain perception recovery (2 dogs) D - OFS evaluation regarding the sub-group (n=20) that had medical discharge within 90 days after FNRIP implementation. – Deep pain perception recovery (4 dogs) Abbreviations: OFS, Open field scale; DPP -, Deep pain negative; 4-AMP, 4-aminopyridine

Supplementary Files

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