

Clinical Studies

The Use of Vitamin B₁₂ Aqua-Acupuncture at BL-40 (*Wei-zhong*) for Treatment of Hindlimb Pain in Canines: A Pilot Study

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ABSTRACT

Hindlimb pain is a common clinical presentation in dogs and may be managed with treatments ranging from pharmaceuticals to integrative therapies such as acupuncture. This pilot study evaluated whether vitamin B₁₂ aqua-acupuncture at BL-40, an important acupoint for hindlimb pain, improved clinical signs in adult dogs. Client-owned dogs with hindlimb pain and a Canine Brief Pain Inventory (CBPI) score >25 on a 0–100 scale were enrolled. Exclusion criteria included cranial cruciate ligament rupture, intervertebral disc disease-related hindlimb pain, lameness associated with neurological deficits, abnormal muscle tone/spasticity, or acupuncture within the previous 6 months. Eligible dogs were randomly allocated to a Test Group (n=18), which received aquapuncture in addition to current treatment, or a Control Group (n=17), which continued current treatment alone. Outcome assessments performed at Weeks 0, 2, 4, 8, and 12 included owner-completed CBPI scores, force plate analysis, range of motion (ROM), lameness scoring, and thigh circumference, with assessors blinded to group assignment. The CBPI pain severity scores improved significantly more in the Test Group, with significant between-group differences at Weeks 2, 4, and 8, and a significant overall group effect across improvement assessments ($p=0.046$). Only the Test Group showed significant improvement in hip ROM. Lameness improved in 27.8% of test dogs versus 5.9% of controls, and thigh circumference increased significantly more in the Test Group when compared to controls. Neither group showed significant improvement in force plate outcomes. These findings suggest that vitamin B₁₂ aqua-acupuncture at BL-40 may provide clinical benefit for dogs with hindlimb pain.

Keywords: aquapuncture, canine, hindlimb pain, traditional Chinese veterinary medicine

ABBREVIATIONS: Aqua-AP: aqua-acupuncture; CBPI: Canine Brief Pain Inventory; IVDD: intervertebral disc disease; NSAIDs: non-steroidal anti-inflammatory drugs; ROM: range of motion; TCVM: traditional Chinese veterinary medicine

Hindlimb pain is a common finding in dogs and is a leading reason for orthopedic consults in small animal practice. In large breed dogs, stifle issues such as cranial cruciate ligament tears and osteoarthritis make up a large proportion of symptomatic patients; however, hip issues resulting in osteoarthritis and back pain are not uncommon.¹ In small breed dogs, back pain and medial patellar luxation resulting in osteoarthritis are more commonly found. Obese dogs and older populations in both large and small breeds are at higher risk for hindlimb pain as a result of any of the aforementioned causes.² Physical examination is required to diagnose hindlimb pain and identify its underlying root cause, with radiographs often used to verify the leading differential diagnosis.

Currently, there are few standard treatment options for hindlimb pain in dogs. Typically, NSAIDs are used to reduce inflammation and provide analgesia for soft tissue or osteoarthritis-associated pain, while steroids may be used when neuropathy is considered the primary cause.³

More recently, integrative approaches are being successfully employed to reduce pain and improve mobility, due to osteoarthritis in dogs. These modalities include physical therapy and rehabilitation, manual manipulation, laser therapy, and autologous platelet injections.⁴

Traditional Chinese veterinary medicine (TCVM) encompasses four modalities (acupuncture, Chinese herbal medicine, food therapy, *Tui-na*) and has been practiced in China for thousands of years. In contemporary Western practice, it is often incorporated into an integrative medical approach and has gained increasing popularity in the United States in recent decades.⁵ TCVM emphasizes identification of the underlying disease process through

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pattern differentiation, allowing development of an individualized, condition-specific treatment plan. In dogs with hindlimb pain, particularly those with osteoarthritis, Bony *Bi* syndrome is a commonly recognized TCVM pattern and is frequently associated with Kidney *Yang* Deficiency or Kidney *Yin* Deficiency.⁶ Clinical signs of Kidney *Yang* Deficiency include difficulty rising and walking, coldness of the back and extremities, lameness that worsens in cold and/or damp weather, and heat seeking. Clinical signs of Kidney *Yin* Deficiency may include arthritis, hind-end weakness, lameness, difficulty rising and walking, cool-seeking behavior, and panting.⁶

Of the four TCVM modalities used in veterinary practice, acupuncture is one of the most common modalities used to treat pain, often serving to decrease inflammation while improving mobility.⁵ Acupuncture points can be stimulated through several techniques, among which the most commonly applied are dry needling with manual manipulation, electro-acupuncture with electrical stimulation through inserted needles, and aqua-acupuncture, which injects a liquid substance into the acupuncture point and creates a localized and prolonged stimulation.⁶ Relative to other acupuncture methods, aquapuncture, especially when limited to a single acupoint, provides a more time-efficient approach and avoids needle retention, which may be poorly tolerated by small animal patients unable to remain still for 10-20 minutes. Although vitamin B₁₂ is a common aquapuncture substrate, other injectables, including polysulfated glycosaminoglycan, sterile saline, and lokivetmab are frequently selected for this acupuncture technique. Commonly used acupoints to treat hindlimb pain and Bony *Bi* syndrome include SP-6, KID-3, BL-21, BL-23, BL-26, BL-40, BL-54, ST-36, GB-21, and *Bai-hui*.

When considering acupoints to treat hindlimb pain, BL-40 (*Wei-zhong*), is important as it is Master point for the caudal back and hip region. This is an important point for removing obstruction in the Bladder Channel, which extends along the length of the spine down to the hind paws. It is therefore an excellent point to consider for a variety of hindlimb disorders, including weakness associated with *Wei* syndrome. Anatomically, BL-40 is located at the center of the popliteal crease between the biceps femoris and semimembranosus muscles, a region that also contains the popliteal lymph nodes and lymphatics responsible for drainage of the distal limb.⁷ Considering the important effect of BL-40 as Master point for the caudal back and hip region, the objective of this study was to evaluate whether aquapuncture with vitamin B₁₂ at this single acupoint could improve clinical signs of hindlimb pain in dogs. It was hypothesized that patients exhibiting hindlimb pain receiving the proposed vitamin B₁₂ aquapuncture at BL-40 would have greater improvements of hindlimb pain/weakness than those receiving no additional treatment.

MATERIALS AND METHODS

Animals

The subject population for this study consisted of canine patients exhibiting hindlimb pain. The subjects were recruited in the southeastern Wisconsin, USA area from

existing clients of the Magnolia Springs Veterinary Center hospital via flyers, email, and Facebook invitation to apply. The clinical study was explained to the owners and with the owner's written consent, any subject that met the following inclusion criteria was enrolled in the study: (1) over the age of two years, (2) exhibiting hindlimb pain, with scores >25 (range 0 to 100) on the validated osteoarthritis pain assessment tool "Canine Brief Pain Inventory (CBPI)," and (3) no acupuncture treatment in the past six months (Figure 1).⁸ Those with diagnosis of ruptured cranial cruciate ligaments in the last six months, hindlimb pain related to IVDD, lameness related to neurological deficits, or with abnormal muscle tone/spasticity, were excluded from study enrollment (Tables 1 and 2). Qualified subjects were evenly assigned to either the Control Group or Test Group using a random sequence generator^a. Subjects in the Test Group received vitamin B₁₂ aquapuncture and those in the Control Group received no

Canine Brief Pain Inventory

Description of pain:
Rate your dog's pain:

1. Fill in the oval next to the one number that best describes the pain at its **worst** in the last 7 days.
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 No pain Extreme pain

2. Fill in the oval next to the one number that best describes the pain at its **least** in the last 7 days.
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 No pain Extreme pain

3. Fill in the oval next to the one number that best describes the pain at its **average** in the last 7 days.
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 No pain Extreme pain

4. Fill in the oval next to the one number that best describes the pain as it is **right now**.
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 No pain Extreme pain

Description of function:
Fill in the oval next to the one number that best describes how during the last 7 days **pain has interfered** with your dog's:

5. **General Activity**
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 Does not interfere Completely interferes

6. **Enjoyment of Life**
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 Does not interfere Completely interferes

7. **Ability to Rise to Standing From Lying Down**
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 Does not interfere Completely interferes

8. **Ability to Walk**
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 Does not interfere Completely interferes

9. **Ability to Run**
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 Does not interfere Completely interferes

10. **Ability to Climb Stairs, Curbs, Doorsteps, etc.**
☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10
 Does not interfere Completely interferes

Overall impression:
11. Fill in the oval next to the one number that best describes your dog's overall quality of life over the last 7 days.
☐ Poor ☐ Fair ☐ Good ☐ Very Good ☐ Excellent

Figure 1: CBPI pain score questionnaire for pain assessment in the study. The questionnaire includes 10 questions (4 for severity and 6 for interference), each scored from 0 (best condition) to 10 (worst condition); therefore, the minimum and maximum a dog could score was 0 and 100, respectively.

Table 1: Neurological grading system used to determine whether a patient's hindlimb pain/lameness was associated with neurological deficits rather than osteoarthritis-associated pain. Dogs with clinically relevant neurological dysfunction were excluded, and enrolled dogs were required to have no more than mildly delayed conscious proprioception. Subjects with scores below 9 or above 18 were excluded.

Neurological Test		Absent	Depressed	Normal	Hyperactive	Hyper with Clonus
Postural Reactions	Conscious proprioception	0	1	2	3	4
	Hopping	0	1	2	3	4
	Extensor postural thrust	0	1	2	3	4
Spinal Reflexes	Ventral sphincter	0	1	2	3	4
	Leg withdrawal	0	1	2	3	4
	Patella	0	1	2	3	4
Sensory Function	Pain perception/nociception	0	1	2	3	4

Table 2: Muscle tone/spasticity assessment used to assess the patient's muscle tone and spasticity. Subjects with abnormal muscle tone or spasticity were excluded as patients with severe atrophy or spasticity would be unlikely to show changes in a short-term study such as this. Subjects with abnormal muscle tone or spasticity were identified when the sum of the two grades was under 4 or over 8.

	Poor	Mild Decrease	Average	Mild Increase	Marked Increase
Muscle Tone	1	2	3	4	5
Spasticity	1	2	3	4	5

additional treatment. To reflect real-world clinical practice, all patients remained on their existing diet and medical therapies, including NSAIDs, joint supplements, and exercise, for the duration of the trial. Owners also agreed not to alter the patient's routine maintenance care during the study and to inform the investigator if any change became necessary, so the change could be documented in the study record or, if clinically significant, the dog could be removed from the study.

Study Design

After enrollment, detailed conventional and TCVM examinations were performed by the author. Following physical examination, the owner moved to a waiting area and the patient moved to the treatment area where a certified veterinary acupuncturist (Dr. Lynn Duppler), either instilled a 50:50 mixture of sterile saline^b and vitamin B₁₂^c at BL-40 bilaterally, or performed no treatment, based on the assigned group. For each subject in the Test Group, the amount of saline diluted vitamin B₁₂ was standardized by body surface area of the patient and was administered with a 27-gauge needle^d. Using body surface area (in m²) as opposed to the bodyweight allowed 0.15 m² intervals to be used rather than set weight intervals, which would better adjust the amount of substrate needed for larger dogs (Table 3). Once the total amount was determined based on the subject's body surface area, the amount was then divided equally between both BL-40 acupoints (Table 4). For example: a patient with a body surface area of 0.4 m² would receive 0.1 mL vitamin B₁₂ (1000 mcg/mL) and 0.1 mL sterile saline, 0.2 mL total, with 0.1 mL of the 50:50 mixture injected at each BL-40 acupoint. Control patients would receive no treatment as inserting a 27-g needle with or without sterile saline (no B₁₂) might induce stimulation of BL-40 and create a response.

Table 3: Conversion of body weight to body surface area

Body Weight (kg)	Body Surface Area (m ²)	Body Weight (kg)	Body Surface Area (m ²)
2	0.16	29	0.95
3	0.21	30	0.98
4	0.25	31	1.00
5	0.30	32	1.02
5.5	0.31	33	1.04
6	0.33	34	1.06
6.5	0.35	35	1.08
7	0.37	36	1.10
7.5	0.39	37	1.12
8	0.40	38	1.14
8.5	0.42	39	1.16
9	0.44	40	1.18
9.5	0.45	41	1.20
10	0.47	42	1.22
11	0.50	43	1.24
12	0.53	44	1.26
13	0.56	45	1.28
14	0.59	46	1.30
15	0.61	47	1.32
16	0.64	48	1.33
17	0.67	49	1.35
18	0.69	50	1.37
19	0.72	51	1.39
20	0.74	52	1.41
21	0.77	53	1.43
22	0.79	54	1.44
23	0.82	55	1.46
24	0.84	56	1.48
25	0.86	57	1.5
26	0.89	58	1.51
27	0.91	59	1.53
28	0.93	60	1.55

Table 4: Quantity of solution (vitamin B₁₂ + saline) for aquapuncture administration according to body surface area

Body Surface Area (m ²)	Vitamin B ₁₂ / Sterile Saline (50:50)
0.15-0.3	0.05 mL each point (0.1 mL total)
0.31-0.45	0.1 mL each point (0.2 mL total)
0.46-0.60	0.15 mL each point (0.3 mL total)
0.61-0.75	0.2 mL each point (0.4 mL total)
0.76-0.80	0.25 mL each point (0.5 mL total)
0.81-0.95	0.3 mL each point (0.6 mL total)
0.96-1.05	0.35 mL each point (0.7 mL total)
1.06-1.20	0.4 mL each point (0.8 mL total)
1.21-1.35	0.45 mL each point (0.9 mL total)
1.36-1.50	0.5 mL each point (1.0 mL total)

After each treatment or no-treatment visit, the patient returned to normal activity at home under owner monitoring. This process was repeated at Weeks 2, 4, 8, and 12, with each patient undergoing examination, treatment or no treatment, and investigator-led data collection at each visit. Owners also completed the CBPI assessment at each study timepoint, resulting in a total trial duration of three months.

Outcome Data Measurement Methods

As the study was double-blinded, with randomization being performed by a member of the technical staff, neither the owners nor the investigator knew which dog received aquapuncture treatment. The following methods were performed on each study dog for assessment and generation of study outcome data.

CBPI Scoring: At each visit, owners completed the CBPI assessment to evaluate pain severity and pain interference associated with their dog's lameness, while the investigator collected clinical data from each study subject. The form was completed by the same person at each assessment, defined as the primary caregiver and the individual most familiar with the dog's daily activity level and clinical signs. Owners were instructed to base their responses on the dog's behavior and mobility since the previous visit. Each item was scored using the standardized CBPI numerical rating scale, with higher scores representing greater pain or functional impairment. Owners were asked to consider routine activities such as rising, walking, running, climbing stairs, jumping, playing, and interacting with family members, as well as lameness-associated changes including stiffness, altered weight-bearing, reluctance to move, and reduced activity. To minimize response bias, owners completed the form independently before discussion with the attending veterinarian. Completed forms were reviewed for missing responses, and clarification was limited to explanation of the form instructions or scoring scale without directing or influencing owner responses.

Force Plate Assessment: Static force plate analysis^e was performed by the author at each visit to objectively assess weight distribution among the limbs. Each dog was

positioned with one paw resting on each sensor area of the force plate and was encouraged to stand squarely and calmly during data collection. The owner stood approximately 4 feet in front of the dog to help maintain head and body alignment and encourage a neutral forward posture. The force plate was positioned at least 5 feet from adjacent walls to minimize postural sway, leaning, or external support. Outcome data generated measured the percentage of body weight borne by each limb. Since the current study aimed to evaluate treatment effects on hindlimb pain, the primary endpoint was calculated as the combined percentage of body weight supported by the two hind limbs, which ideally approximates 40% in normal dogs.

Range of Motion Assessment (ROM): Hip and stifle ROM were measured using a goniometer^f at Week 0, prior to treatment, and again at study termination (Week 12). For a better assessment on the treatment effect for both hip and stifle ROM data, the side (either left or right) with the worse ROM outcome at Week 0 was chosen from each subject to determine the treatment effect on ROM improvement observed at Week 12. All ROM measurements were performed with the patient in lateral recumbency to improve consistency among assessments and to maintain patient comfort; particularly to accommodate dogs that had difficulty standing on one limb, while the contralateral leg was measured, due to pain. The owner remained with the dog throughout the assessment, and each patient was positioned in the manner that provided the greatest comfort while still allowing standardized measurement. A goniometer was placed with its center point (fulcrum), over the joint being measured (hip or stifle), and the two arms were aligned with specific bony landmarks above and below the joint.

For the hip measurement, the fulcrum was placed over the greater trochanter of the femur. One arm was aligned using the ilium or a line toward the lumbosacral region as the proximal reference. The other arm was aligned along the femur toward the lateral femoral condyle. Flexion and extension angles were measured and hip ROM was calculated as the difference between extension and flexion angles. A larger calculated value indicated greater hip mobility. For the stifle measurement, a single goniometric measurement was recorded for each limb rather than a calculated flexion-extension range. This value represented the measured stifle angle at maximal comfortable flexion; therefore, a smaller angle indicated greater stifle flexion and improved mobility.

Lameness Scoring: Lameness was evaluated and graded for each dog at Week 0, before treatment, and again at study termination (Week 12). A study dog walked alongside the owner on leash both away and towards the author for a length of 30 feet. This was then repeated at a trot. Lameness was graded using a 0 to 5 scale, where 0=normal gait at walk and trot, 1=slight lameness, 2=obvious weight-bearing lameness, 3=severe weight-bearing lameness, 4=intermittent non-weight-bearing lameness, and 5=continuous non-weight-bearing lameness.

Thigh Circumference Assessment: Thigh circumference was measured at Week 0, before treatment, and again at study termination (Week 12) using a Gulick tape^g measure.

Measurements were performed with the dog standing squarely, with weight evenly distributed on all limbs (as tolerated by the patient). Owners were positioned in front of the dog to encourage forward attention and reduce leaning or swaying to one side during measurement. For each dog, the pelvic limb with the smaller thigh circumference at baseline was selected for subsequent analysis, using the same approach applied to the ROM analysis to evaluate change in the more clinically affected limb. Thigh circumference was measured at a standardized location (midpoint between the greater trochanter and lateral femoral condyle, perpendicular to the femur). The same site was used for all repeat measurements. The Gulick tape was positioned perpendicular to the long axis of the femur and wrapped snugly around the thigh without compressing the soft tissues. The tape was pulled until the built-in tension indicator reached the standardized mark, and circumference was recorded in centimeters. Each measurement was repeated three times, and the mean value was used for analysis.

Statistical Analysis

Given the pilot nature and small sample size of the study, data analysis focused on descriptive statistics, including means, standard deviations, and percentage differences, to identify emerging trends for future large-scale clinical trials. Nonetheless, commonly applied statistical inference methods were applied to determine if the observed trends were statistically significant. For instance, one-sided two-sample *t* test (when normality held) or Wilcoxon rank sum test (when normality did not hold) was applied to compare two groups with respect to the mean/median for a single-time assessment. Two-group repeated-measure ANOVA (when normality held) or the nonparametric aligned rank transform ANOVA (when normality did not hold) test was used to assess the group effect over time. Significance level for all tests was set at 0.05. The open-source statistical software R^h was used for all statistical analyses.

RESULTS

A total of 37 dogs with hindlimb pain and meeting the inclusion/exclusion criteria were enrolled in the study, among which 18 dogs were randomly assigned to the Control Group and the remaining 19 were in the Test Group. Two subjects had to be removed from the study prior to completion: 1) one dog after Week 2, when an osteosarcoma was diagnosed and the subject was euthanized shortly thereafter; 2) the second dog after Week 8, when a splenic mass was diagnosed and the owner voluntarily withdrew the subject after surgery. All remaining subjects (*n*=35) completed the 12-week study.

None of the signalment parameters (sex proportion, age, body weight) as well as the muscle tone/spasticity score and neuro-grading showed significant group differences; although age difference approached significance (controls 11.3 years, test 9.2 years, *p*=0.051). Both groups included a variety of breeds, with a total of 16 breeds in the Control Group and 17 in the Test Group (Table 5).

CBPI Scores Analysis

The mean values of pain severity score within each treatment group over the five assessment times were analyzed (Figure 2; Table 6). At Week 0 (baseline time), the between-group difference was not statistically significant (*p*=0.645), suggesting group comparability on the initial pain severity score. At Week 2 and Week 4 post-treatment assessments, the Test Group showed significant improvement, whereas the Control Group demonstrated worsening severity scores relative to its Week 0 mean. At Week 8 and Week 12, the improvements in the Test Group were 314% and 84.2%, respectively, greater than those in the Control Group. The between-group differences were statistically significant at Weeks 2, 4, and 8. When all 4 improvement assessments were considered, ANOVA revealed a significant overall group effect across improvement assessments (*p*=0.046), indicating greater improvement in pain severity scores in the Test Group.

Table 5: Summary statistics of signalment data, neurological score, and muscle tone / spasticity assessment

	Control Group	Test Group	<i>p</i> -value
Sex Proportion	Female: 29.4% Male=70.6%	Female: 55.6% Male=44.4%	0.176
Age (years)	11.3±3.0	9.2±3.2	0.051
Weight (lbs)	51.4±29.5	66.8±40.2	0.205
Muscle Tone / Spasticity Score	5.5±0.6	5.4±0.6	0.356
Neuro Grading	13.2±1.0	13.1±1.4	0.838
Breeds	Aussie-doodle, Australian Shepherd Mix, Boxer Mix, Cocker Spaniel, Dachshund, Dutch Shepherd, English Shepherd, German Shepherd, Labrador, Labrador Mix, Miniature Pincher Mix, Newfoundland, Pekingese, Poodle Mix, Terrier Mix, and Weimaraner	English Mastiff, Australian Shepherd Mix, Beagle Mix, Border Collie Mix, Dachshund Mix, Doberman Pinscher Mix, English Shepherd, German Shepherd, Golden Retriever, Italian Greyhound, Lab Shepherd Mix, Labrador, Mastiff, Olde English Bulldog, Pit Bull, Shih Tzu, and Wheaton Terrier Mix	—

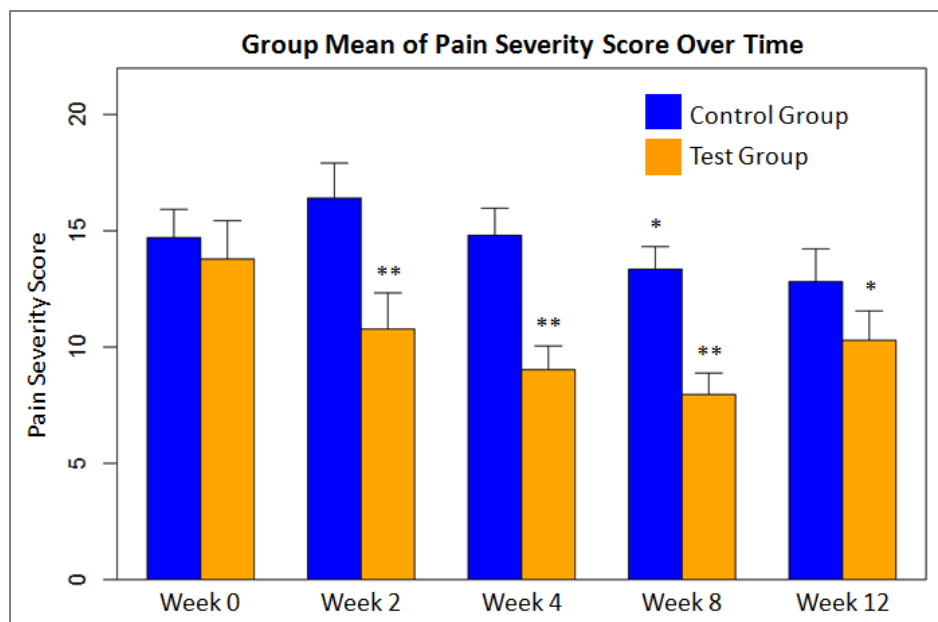


Figure 2: Group mean of CBPI severity score and comparisons between groups. * $p < 0.05$; ** $p < 0.01$ compared with Week 0 within the group.

Table 6: Summary statistics (mean±SD) of CBPI pain severity score data

Group	Week 0	Week 2	Week 4	Week 8	Week 12
Control	14.7±5.1	16.4±6.2	14.8±4.7	13.4±4.0	12.8±5.7
	Improvement from Week 0	-1.7±7.1 (↓11.6%) ($p_i=0.831$)	-0.1±4.4 (↓0.7%) ($p_i=0.543$)	1.4±3.1 (↑9.5%) ($p_i=0.044$)*	1.9±5.9 (↑12.9%) ($p_i=0.105$)
Test	13.8±7.0	10.8±6.6	9.1±4.2	7.9±4.0	10.3±5.4
	Improvement from Week 0	3.0±4.0 (↑21.7%) ($p_i=0.003$)**	4.7±6.0 (↑34.1%) ($p_i=0.002$)**	5.8±7.9 (↑42.0%) ($p_i=0.003$)**	3.5±8.2 (↑25.4%) ($p_i=0.044$)*
p -value	$p_0=0.645$	$p_2=0.012$ *	$p_2=0.005$ **	$p_2=0.018$ *	$p_2=0.253$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. p_0 : baseline (Week 0) group comparison; p_i : improvement compared to Week 0 within group; p_2 : improvement comparison between groups. Same notations for Tables 7-11.

For pain interference scores, no significant between-group difference was observed at Week 0 ($p=0.277$). The Test Group showed greater mean improvement than the Control Group at post-treatment assessments, with improvements of 94.1%, 78.4%, 83.1% and 53.3% at

Weeks 2, 4, 8, 12, respectively; when compared with the corresponding mean improvements in the Control Group (Figure 3; Table 7). Despite the large numerical differences, the combined ANOVA analysis of the four improvement outcomes was unable to reach a statistically significant between-group effect ($p=0.186$) in this small pilot study.

Table 7: Summary statistics (mean±SD) of CBPI pain interference score data

Group	Week 0	Week 2	Week 4	Week 8	Week 12
Control	26.7±10.2	23.4±9.7	21.6±10.3	20.8±8.0	22.2±13.1
	Improvement from Week 0	3.4±8.6 (↑12.7%) ($p_i=0.063$)	5.1±10.5 (↑19.1%) ($p_i=0.030$)*	5.9±7.5 (↑22.1%) ($p_i=0.003$)**	4.5±9.0 (↑16.9%) ($p_i=0.029$)*
Test	22.8±10.5	16.2±11.8	13.7±7.6	12.1±6.8	15.9±10.0
	Improvement from Week 0	6.6±9.1 (↑28.9%) ($p_i=0.002$)**	9.1±10.5 (↑39.9%) ($p_i=0.0009$)**	10.8±11.5 (↑47.4%) ($p_i=0.0005$)**	6.9±11.7 (↑30.3%) ($p_i=0.015$)*
p -value	$p_0=0.277$	$p_2=0.249$	$p_2=0.134$	$p_2=0.072$	$p_2=0.581$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

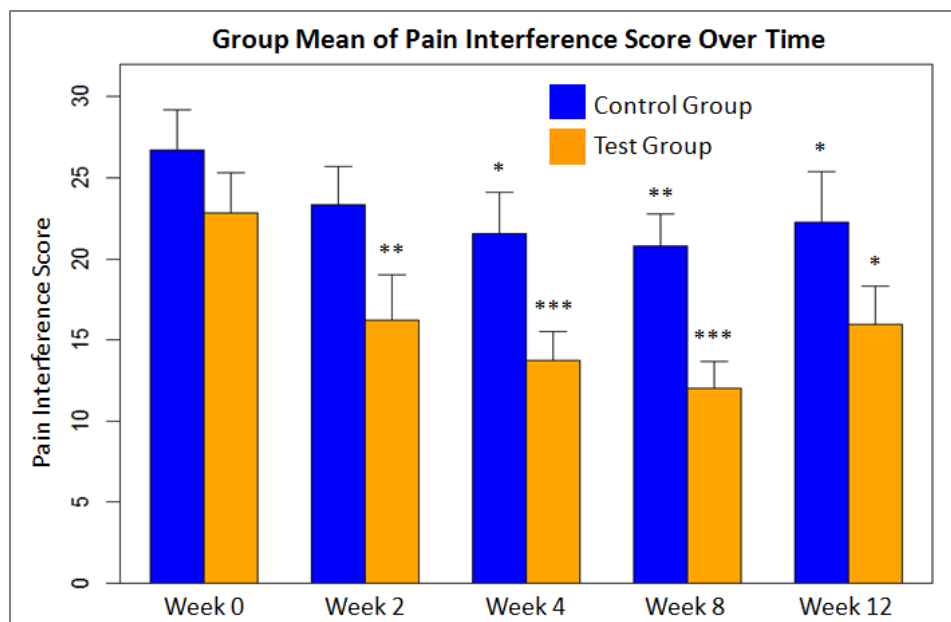


Figure 3: Group mean of CBPI interference score and comparisons between groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with Week 0

Force Plate Analysis

At baseline (Week 0), both groups demonstrated lower-than-ideal mean values (Control: 37.8%; Test: 36.8%), with no significant difference between groups ($p = 0.713$) (Table 8). In the Test Group, hindlimb weight-bearing increased from 36.8% at baseline to 39.8% at Week 2, almost reaching ideal percent weight bearing which approached statistical significance ($p = 0.056$). This magnitude of improvement, however, was not sustained at later timepoints, with a

Week 12 mean of 38.3% (still 1.5% higher than baseline). The Control Group showed a smaller increase from 37.8% to 38.4% at Week 2 that was not statistically significant ($p = 0.312$); and also had slight improvement (0.8%) at Week 12 when compared to baseline. Overall, no statistically significant changes were reached across study timepoints within either group, and no significant differences were observed between treatment groups.

Table 8: Summary statistics (mean±SD) of force plate data (% of body weight on hind limbs)

Group	Week 0	Week 2	Week 4	Week 8	Week 12
Control	37.8±14.7	38.4±6.2	38.4±7.2	36.9±6.5	38.6±4.7
	Improvement from Week 0	0.6±5.3 (↑1.6%) ($p_1=0.312$)	0.6±6.3 (↑1.6%) ($p_1=0.342$)	-0.8±4.2 (↓2.1%) ($p_1=0.785$)	0.8±5.1 (↑2.4%) ($p_1=0.252$)
Test	36.8±6.6	39.8±6.9	37.8±6.8	34.6±4.7	38.3±9.2
	Improvement from Week 0	3.1±7.7 (↑8.4%) ($p_1=0.056$)	1.0±8.8 (↑2.7%) ($p_1=0.318$)	-2.2±4.4 (↓6.0%) ($p_1=0.975$)	1.5±10.4 (↑4.1%) ($p_1=0.425$)
p-value	$p_0=0.713$	$p_2=0.142$	$p_2=0.445$	$p_2=0.816$	$p_2=0.685$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Range of Motion Analysis

Hip and stifle ROM were evaluated at Week 0 and Week 12 (Table 9). To more accurately assess treatment effect, the limb with the poorer ROM at baseline (Week 0) was selected for each subject for both hip and stifle analyses at Week 12. At Week 0, baseline hip ROM did not differ significantly between groups ($p = 0.099$). Both groups demonstrated improvement in hip ROM by Week 12. In the Control Group, the mean hip ROM improved from 93.6 to 95.1 (1.6% increase, $p = 0.38$), whereas the Test Group improved from 84.6 to 93.6 (10.6% increase, $p = 0.008$).

Despite this within-group improvement, direct comparison of mean change between groups was unable to reach statistical significance in this small study ($p = 0.098$). Similarly, baseline stifle ROM did not differ significantly between groups at Week 0 ($p = 0.506$). By Week 12, both groups showed similar statistically significant improvement in stifle ROM (Control: 8.2% improvement, $p = 0.029$; Test: 6.2% improvement, $p = 0.043$). Comparison of mean between-group improvement revealed no significant difference ($p = 0.638$).

Table 9: Summary statistics and statistical inference on ROM data

Group	Hips		Stifles	
	Week 0	Week 12	Week 0	Week 12
Control	93.6±10.7	95.1±13.8	44.9±6.5	41.2±7.7
	Improvement from Week 0	1.5±18.5 (↑1.6%) ($p_1=0.380$)	Improvement from Week 0	3.7±7.5 (↑8.2%) ($p_1=0.029^*$)
Test	84.6±16.6	93.6±14.3	46.7±9.5	43.8±11.6
	Improvement from Week 0	9.0±14.2 (↑10.6%) ($p_1=0.008^{**}$)	Improvement from Week 0	2.9±5.9 (↑6.2%) ($p_1=0.043^*$)
<i>p</i> -value	$p_0=0.099$	$p_2=0.098$	$p_0=0.506$	$p_2=0.638$

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

Table 10: Distribution of dogs in the Control and Test Groups according to lameness score at Weeks 0 and 12

Week 0						
Group	Score=0	Score=1	Score=2	Score=3	Score=4	Score=5
Control (n=17)	0	14	3	0	0	0
Test (n=18)	0	13	3	1	1	0
Week 12						
Group	Score=0	Score=1	Score=2	Score=3	Score=4	Score=5
Control (n=17)	0	12	5	0	0	0
Test (n=18)	3	11	2	2	0	0

Lameness Score Analysis

At Week 0, most subjects in both groups had a lameness score of 1 (Table 10). After 12 weeks, only 1 of 17 dogs (5.9%) in the Control Group demonstrated improvement in lameness score, while 3 dogs worsened. In contrast, 5 of 18 dogs (27.8%) in the Test Group showed improvement, and none experienced worsening. The improvement rate in the Test Group was 4.72 times that of the Control Group. This difference did not reach statistical significance in this small study ($p=0.101$).

To enhance baseline comparability, an additional analysis was performed including only subjects with an initial lameness score of 1 or 2. Within this subset, 1 of 17 dogs (5.9%) in the Control Group improved (3 worsened), whereas 4 of 16 dogs (25.0%) in the Test Group improved, with no dogs worsening. Although the improvement rate in the Test Group remained higher, 4.25 times that of the Control Group, the between-group difference again was unable to reach statistical significance ($p=0.130$) in this small study.

Thigh Circumference Analysis

Using the same approach applied in the ROM analysis to better assess treatment effect, the limb with the smaller (weaker) thigh circumference at Week 0 was selected for each subject. Changes observed at Week 12 were then evaluated relative to this baseline measurement (Table 11).

At baseline, thigh circumference did not differ significantly between groups ($p=0.275$). After 12 weeks, contrasting trends were observed. The Control Group demonstrated a slight reduction (3.1%) in thigh circumference, which was not statistically significant ($p=0.884$). In contrast, the Test Group showed an increase (2.7%) in thigh circumference at Week 12, approaching statistical significance ($p=0.052$). Importantly, direct comparison of the mean change between groups concluded a statistically significant between-group difference ($p=0.025$), for improvement of thigh circumference in the Test Group.

Table 11: Summary statistics and statistical inference of thigh circumference data

Group	Week 0	Week 12
Control	26.2±7.9	25.4±6.9
	Improvement from Week 0	-0.8±2.6 (↓3.1%) ($p_1=0.884$)
Test	29.3±8.4	30.1±7.9
	Improvement from Week 0	0.8±2.1 (↑2.7%) ($p_1=0.052$)
<i>p</i> -value	$p_0=0.275$	$p_2=0.025^*$

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

DISCUSSION

This randomized, double-blind controlled trial evaluated whether aquapuncture using vitamin B₁₂ at BL-40 could improve clinical signs of hindlimb pain in dogs. Outcome measures included both owner-reported CBPI scores (pain severity, pain interference) and investigator assessments (lameness, hip and stifle ROM, thigh circumference, force plate analysis) collected over a 12-week period. Owner-reported CBPI data demonstrated consistent and significant reductions in pain severity in the Test Group at all post-treatment timepoints (Weeks 2, 4, 8, and 12), whereas the Control Group demonstrated only a single significant transient improvement at Week 8. Pain interference scores achieved statistical significance for both study groups, however, the BL-40 aquapuncture group demonstrated significant improvements of greater magnitude and consistency when compared to controls. Overall, these findings suggest that BL-40 aquapuncture contributed to reductions in owner-perceived pain and its functional impact.

Investigator-assessed measures provided additional, though somewhat more variable, support for the positive clinical trend observed in owner-reported CBPI scores. Hip ROM improved significantly only in the Test Group, whereas stifle ROM improved significantly in both groups, with no clear between-group difference. Although statistical significance was not achieved in this small, underpowered study, lameness scores suggested a clinically favorable pattern: dogs in the Test Group were more than four times as likely to improve, and none worsened during the 12-week study period, while several dogs in the Control Group exhibited worsening lameness. Thigh circumference increased in the Test Group and declined slightly in controls, resulting in a significant between-group difference favoring the Test Group, suggesting improved limb use. Early force plate measurements suggested greater improvement in the Test Group, approaching statistical significance at Week 2 ($p=0.056$); however, this effect was not sustained at subsequent time points when treatment frequency was reduced, and no statistically significant differences were observed thereafter. Taken together, the combination of owner-reported pain reduction, improved joint mobility, and favorable lameness trends suggests clinically meaningful benefits associated with BL-40 aquapuncture for dogs with hindlimb pain.

Aqua-acupuncture, which involves injection of substances such as vitamin B₁₂ into an acupoint, is thought to enhance acupuncture stimulation through both mechanical and biochemical mechanisms. Injection of a liquid substrate alters the local spatial configuration of tissues while simultaneously stimulating sensory receptors within the acupoint, thereby activating neural pathways.⁹ Experimental work has demonstrated that such stimulation can trigger neuronal activation within the spinal cord, reflected by increased expression of immediate-early gene transcription factors such as Fos protein in the spinal dorsal horn. Fos expression is widely used as a marker of neuronal activation and has been shown to increase following acupuncture stimulation. Studies examining this relationship

at acupoints, such as ST-36 (L3-L5 innervation area), have demonstrated activation of neurons in the corresponding spinal segments, suggesting that acupoint stimulation may influence neural circuits associated with innervation of the stimulated region.⁹ A similar mechanism may be relevant for Bladder 40 (BL-40), which lies in the popliteal region near the branching of the sciatic nerve (L6-S2) into the tibial and common fibular nerves that innervate the extensors of the hock and flexors of the stifle.⁷ Stimulation at this location could influence neural pathways associated with hindlimb pain and function and may provide a neuroanatomical explanation for the improvement in hindlimb weight bearing observed in the present study.

To the best of the authors' knowledge at the time of this report, no published studies have specifically evaluated the isolated effects of BL-40 acupuncture on hindlimb pain in dogs. A recent study by Payne reported that aquapuncture using a commercial polysulfated glycosaminoglycan (Adequan®) administered at BL-11 and BL-23 resulted in greater clinical improvement than standard intramuscular injection in dogs with osteoarthritis.¹⁰ In traditional Chinese medicine, BL-11 is the Association (Back-shu) point for bone, while BL-23 is the Association point for Kidney, which is considered to govern bone health. These points, like BL-40, are commonly incorporated into treatment protocols for osteoarthritis. BL-40 is more specifically associated with disorders of the lower back and pelvic limbs, whereas BL-11 and BL-23 are typically applied for broader musculoskeletal or generalized osteoarthritic conditions. Dogs in Payne's study demonstrated improvement in pain severity scores, supporting the potential clinical relevance of aquapuncture at Bladder Meridian points for musculoskeletal pain. Similar methodological considerations were present in both studies, including heterogeneity in pretreatment clinical conditions and relatively low baseline CBPI scores in some patients, limited the measurable magnitude of improvement and reduced the statistical strength of the observed treatment effects. Together, these findings suggest that aquapuncture at clinically relevant Bladder Meridian points, including BL-40, may contribute to improvements in osteoarthritis-associated pain, although additional controlled studies are needed to further clarify acupoint-specific treatment effects.

Although not all outcome measures demonstrated a significant treatment effect compared with controls, the findings remain encouraging, particularly with respect to pain relief. Aquapuncture provides more prolonged acupoint stimulation than traditional dry needling, which may contribute to greater or more sustained analgesic effects. In the present study, the greatest magnitude of improvement in hindlimb pain measures occurred early in the treatment period, while greater variability was observed later as treatment frequency decreased from two week to monthly sessions. This pattern is consistent with acupuncture dosing literature, which suggests that treatment frequency can influence clinical outcomes, with more frequent sessions typically recommended during periods of active musculoskeletal pain and longer intervals used once maintenance therapy is initiated.¹¹ Similarly, a large individual patient meta-analysis conducted by Vickers and

colleagues reported improved pain outcomes with a greater number of needles and treatment sessions, emphasizing the importance of treatment “dose.”¹² The reduction in treatment frequency later in the study may therefore have limited the magnitude or persistence of improvement in hindlimb pain observed early in the study test population.

As a pilot study, one of the primary values of this trial was its ability to identify protocol-related weaknesses and sources of variability that should be addressed before conducting a larger, adequately powered clinical trial. Limitations recognized in the present study may have influenced interpretation of the results, but these findings provide important guidance for improving future study design. First, the trial was conducted in a moderate-volume small animal practice, and to achieve adequate enrollment, dogs with a wider range of ages, chronicity of disease, and mobility concerns were accepted. Patients were also allowed to remain in the study when unrelated medical conditions were identified and treated. This approach likely improved the real-world applicability of the study population, but it also increased variability among subjects and may have reduced the ability to detect statistically significant between-group differences. In addition, dogs remained on their current medications and supplements, with or without dose adjustment, which created the possibility that some observed improvement reflected concurrent conventional therapies or supportive treatments rather than aquapuncture alone.

Objective assessment of pain in veterinary patients also presented challenges. Although evaluators were blinded, owner-reported CBPI scores showed variability, likely reflecting differences in owner perception of pain and functional impairment. In some cases, owner-reported scores did not align with clinical impressions or verbal reports of improvement, highlighting the subjective nature of this measure. Conversely, some highly observant owners detected and documented subtle gait changes following treatment and were able to correctly predict which treatment group their dog was in. Additionally, detection of pain was challenged by baseline differences between groups which may have limited interpretation of lameness outcomes. At Week 0, the Test Group included 1 dog with grade 3 lameness and 1 dog with grade 4 lameness, whereas no Control Group dogs had lameness of this severity. In a small pilot study, this imbalance created greater opportunity for improvement in the Test Group, including the possibility that dogs with more severe baseline lameness could improve to a level comparable with controls.

The ROM analysis also revealed an opportunity to strengthen the protocol. For stifle and hip ROM measurements, both hind limbs received aquapuncture; therefore, including both treated limbs in the analysis may have provided a more neutral assessment of treatment effect. Analyzing only the more severely affected side may have introduced bias toward detecting improvement because the most affected limb has greater potential for measurable change. Other limitations included the absence of a sham or non-acupoint vitamin B₁₂ control. The possibility cannot be excluded that some clinical improvement was related to systemic or local effects of vitamin B₁₂,

particularly in older dogs. Finally, the small sample size, measurable improvement in some control dogs, and inherent measurement variability may have reduced statistical power across several outcome measures, including pain interference, ROM, lameness, and force plate analysis. Factors such as patient cooperation, examiner technique, and habituation to chronically altered stance may have further contributed to variability. These limitations are consistent with the exploratory nature of a pilot study and underscore the importance of using the findings to refine future protocols.

The limitations identified in this pilot study provide a practical framework for strengthening a future clinical trial. Recruiting a larger patient population would allow narrower inclusion criteria, more uniform case selection, and improved statistical power. Subject variability could be reduced by limiting enrollment to dogs within a smaller age range, dogs with a defined orthopedic condition such as partial cranial cruciate ligament rupture, or dogs evaluated at a standardized time point (e.g. 3 months after TPLO surgery). A larger study population would also permit removal of subjects that develop unrelated medical conditions during the study period, thereby reducing potential confounding effects.

Refinement of outcome measures and statistical methods may further improve study design. Consideration should be given to including both treated limbs in ROM analyses or using statistical methods that account for paired measurements within the same dog. While force plate analysis is valuable for quantifying lameness and weight-shifting, it may be less sensitive as a direct measure of chronic pain, particularly in dogs that have developed compensatory stance patterns after months of discomfort. Study quality could also be improved by increasing the consistency and sensitivity of CBPI assessments; possibly by recruiting more observant owners who are accustomed to evaluating subtle changes in gait and comfort, such as those involved in agility or other structured canine activities. A subsequent clinical trial should also consider maintaining treatment at 2-week intervals, rather than transitioning to monthly maintenance therapy. This may better address chronic pain, as the shift to maintenance treatment in the present study may have occurred prematurely. Finally, this research framework could also be adapted to evaluate other injectable substrates used in the management of osteoarthritis. For example, a similar double-blind study could compare conventional administration of polysulfated glycosaminoglycans with aquapuncture at BL-40, using comparable outcome measures.

In conclusion, although not all endpoints reached statistical significance, consistent improvement trends were observed across multiple outcome measures, including reductions in owner-reported pain, improved hip mobility, and favorable lameness trends. By providing preliminary evidence of potential clinical benefit and identifying key methodological limitations, this pilot study establishes a foundation for a larger, adequately powered clinical trial with a refined protocol designed to minimize variability, address confounding factors, and more clearly define the clinical role of this treatment approach.

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FOOTNOTES

- ^a <https://www.random.org/sequences/>
^b Saline solution 0.9% sterile, Phoenix Pharmaceutical Inc, Legaspi Village, Makati City, Philippines
^c Vitamin B₁₂ injection (Cyanocobalamin) 1000 mcg/mL, Phoenix Pharmaceutical Inc, Legaspi Village, Makati City, Philippines
^d Exel hypodermic disposable needles, EXELINT International Co, Baldwin Park, CA, USA
^e Stance analyzer, Companion Animal Health, Lewisville, Texas, USA
^f Baseline goniometer 6", 8" and 12", Fabrication Enterprises Inc, White Plains, NY, USA
^g Gulick spring tape measure - 6 ft, Blue Jay an Elite Healthcare Brand, Middletown, NY, USA
^h R version 4.4.1. The R Foundation for Statistical Computing, Vienna Austria; <http://www.R-project.org>

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