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Clinical Studies

Effects of Acupuncture at PC-6 and LU-9 on Blood Flow in Healthy Dogs Based on Ultrasound Color Doppler Imaging

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ABSTRACT
Pulse diagnosis is an important technique in traditional Chinese (veterinary) medicine, wherein acupuncture (AP) treatment is adjusted according to pulse changes (blood flow). This diagnosis, however, can be subjective. Currently no quantitative evaluation method of blood flow secondary to acupoint stimulation has been established. The objective of this research was to apply ultrasound color Doppler imaging (UCDI) to assess the effect of AP at LU-9 and PC-6 on blood flow. Thirty-two healthy dogs were enrolled, each receiving five different AP treatments (≥1 week apart): AP at LU-9; AP at PC-6; AP at LU-9 and PC-6; AP at a sham point; and no AP (waiting in room). Before and after each treatment, the same investigator, blinded to AP treatment, performed UCDI to record blood flow data. Outcome data included vessel diameter (VD), peak systolic velocity (PSV), end-diastolic velocity (EDV), time average maximum velocity (TAMAX), and pulsatility indices (PI). Additionally, blood flow calculated endpoints included: resistive index (RI), cross-sectional area (CSA), and blood flow volume (BFV). The data showed that AP at PC-6 had a statistically significant impact on blood flow parameters: VD (p=0.002), PSV (p=0.001), EDV (p=0.005), TAMAX (p<0.001), CSA (p=0.005), and BFV (p<0.001). The findings from this study suggest that AP treatment could increase blood flow, especially with respect to flow velocity and volume, dependent on acupoint used. Clinical application of the Doppler ultrasound technique used in this study, allows monitoring of acupuncture-associated blood flow changes, leading to development of unique therapeutic approaches to manage canine vascular and cardiac diseases.

Keywords: acupuncture, blood flow, dogs, pulse diagnosis, ultrasound color Doppler imaging

ABBREVIATIONS: AP: acupuncture; BFV: blood flow volume; CSA: cross-sectional area; CTA: computed tomography angiography; EDV: end-diastolic velocity; LDF: laser Doppler flowmetry; PAD: peripheral arterial disease; PI: pulsatility indices; PSV: peak systolic velocity; RI: resistive index; TAMAX: time average maximum velocity; TCM: traditional Chinese medicine; TCVM: traditional Chinese veterinary medicine; UCDI: ultrasound color Doppler imaging; VD: vessel diameter

Blood flow refers to the movement of blood through the circulatory system, which includes the heart, arteries, veins, and capillaries. It is an essential physiological process that delivers oxygen and nutrients to cells and tissues throughout the body while removing waste products like carbon dioxide. Clinical applications of blood flow assessment are vital in both human and veterinary medicine. From diagnosing cardiovascular diseases, ischemic stroke and peripheral vascular disease, to managing wound care, intraoperative blood flow, transplanted organs and optimizing drug delivery; understanding blood flow dynamics is crucial for effective medical care.[1-8] The advancements in imaging and monitoring techniques have significantly enhanced the ability to evaluate and manage blood flow, improving patient outcomes across various medical fields.

Important parameters of blood flow commonly measured and monitored in clinical practice include blood flow velocity (or blood flow rate), pulse waveform analysis, resistance and compliance, pulsatility index, and resistance index.[9-12] Methods for measuring blood flow include: Doppler ultrasound, magnetic resonance imaging (MRI), perfusion imaging, computed tomography angiography (CTA), laser Doppler flowmetry (LDF), and ultrasound color Doppler imaging (UCDI).[13-18] Although each method has its merits, the choice of UCDI for this study is based on its non-invasiveness, real-time capabilities, and the specific focus on understanding the impact of acupuncture (AP) on blood flow in small animals without the need for general anesthesia. The continuous advancements and positive reviews regarding UCDI's clinical applications make it a suitable and promising tool for research on blood flow.

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In traditional Chinese medicine (TCM) and traditional Chinese veterinary medicine (TCVM), acupuncture needles are inserted at specific anatomic points that may correspond to areas in close proximity to blood vessels or nerves. The stimulation of these acupoints is believed to have both local and systemic effects. Locally, acupuncture (AP) may cause vasodilation and increased blood flow to the surrounding tissues. Systemically, it is thought to influence the circulatory system, promoting improved blood circulation throughout the body.[19]

The acupuncture point, PC-6 (Pericardium 6 or *Nei-guan*), is believed to have specific effects on the pulse and blood vessels, which is deeply rooted in TCM/TCVM theory[20,21] Hence, it has been postulated that stimulating PC-6 can change blood flow of patients, although this has not been demonstrated. Furthermore, the AP point, LU-9 (*Tai-yuan*), is considered the "Influential point for pulse" and is believed to have an influence on the pulse and blood vessels; however, scientific evidence for this claim also remains lacking so far.[22]

The AP points PC-6 and LU-9 were chosen to study AP's effects on blood-flow in dogs (Figure 1). It is postulated that changes of blood flow by PC-6 stimulation or LU-9 stimulation would be visible on color Doppler ultrasound, a non-invasive imaging technique that uses sound waves to create a two-dimensional image of blood vessels and blood flow. The objective of the study was to determine the effects of performing AP at acupoints PC-6 and/or LU-9 on the blood flow of healthy dogs based on the analysis of UCDI data. With a randomized repeat-measure controlled trial, the study hypothesized that the changes in blood flow parameters after receiving AP treatment at PC-6 and/or LU-9 would be greater than the changes after the sham or no AP treatment sessions.

MATERIALS AND METHODS

Subject Population

The subject population consisted of clinically healthy dogs, with no sign of disease or on any medication, regardless of age, breed, or sex. Subjects were recruited and enrolled from two sources: (1) animal shelters; and (2) dogs that visited the primary investigator's (YC) clinic. Only those with consent from their caretakers, and who committed for 5 visits, were enrolled in the study. There were no exclusion criteria.

Intervention and Data Collection

Since the blood flow change due to AP stimulation was expected to be temporary (i.e. should return to the baseline after a washout period); to be more efficient, the study employed a repeated measure (or randomized complete block where each subject was a block) design. Each subject received the following 5 different AP treatments in a randomized order: A) receive AP at LU-9; B) receive AP at PC-6; C) receive AP at both LU-9 and PC-6; D) receive AP at a sham point; E) no treatment (wait for 10 minutes in the room). Each of the 5 AP sessions had at least 1 week between visits to wash out possible residual effects.

All AP sessions lasted for 10 minutes; in session E, the subject waited in the same room for 10 minutes. The random order for each subject was determined using the following method: each subject was assigned one envelope which contained 5 pieces of paper that had written A, B, C, D, or E. One piece of paper was randomly drawn at each visit. The 5 visits and the respective AP treatment were recorded in each subject's "Visit Record and AP Trial" sheet, which was not revealed to the primary investigator, who performed the UCDI procedure.

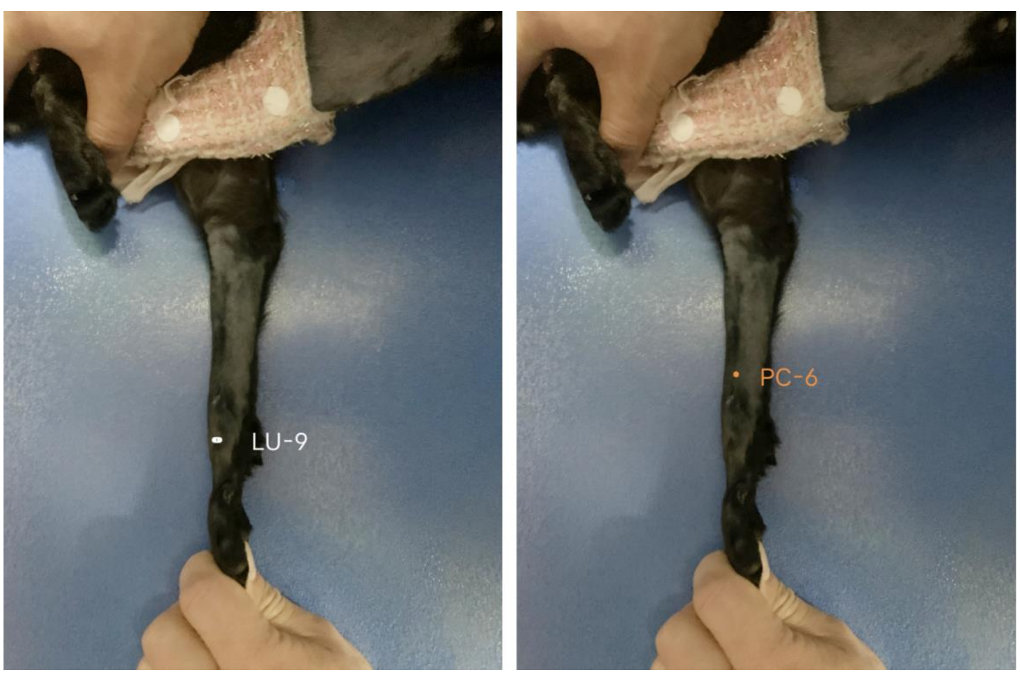

Figure 1: The picture on the left shows the location of LU-9 which is on the medial aspect of the radiocarpal joint just cranial to the radial artery; the picture on the right shows the location of PC-6 in study dogs which is on the medial side of the thoracic limb, 3 *cun* proximal to the carpal crease in the groove between the flexor carpi radialis and the superficial digital flexor muscles.

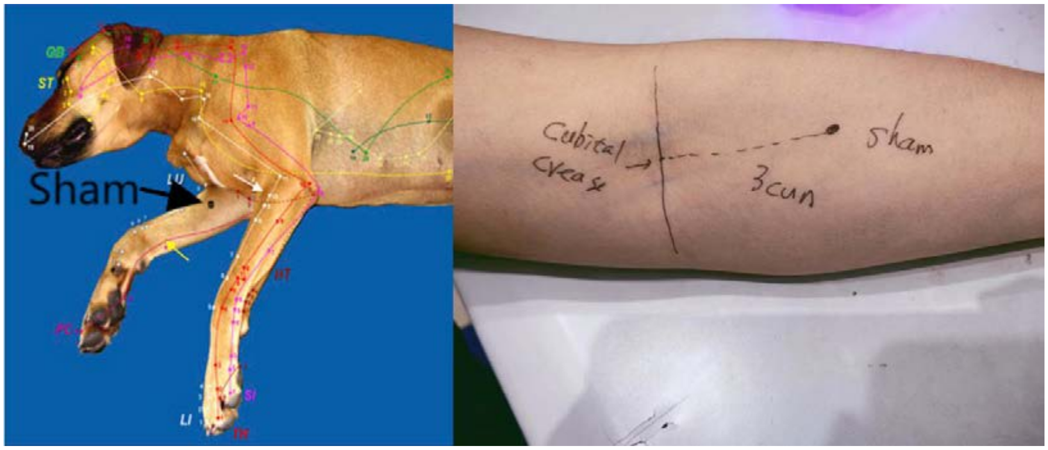

Figure 2: The picture on the left shows the Regular Channels containing acupoints coursing along a dog's front legs. In addition, it shows the sham point (black point with black arrow) used in the study. The sham point was located on the front leg 3 *cun* away from the middle point of the cubital crease, with 1 *cun* depth; and not located on the 12 Regular Channels. The picture on the right is of a human arm demonstrating greater detail of the sham point location.

For each AP treatment, single-use sterile AP needles[a] (0.30×25 mm/30-gauge, 1 inch length) were used for all dogs. The depth of stimulation at LU-9 (bilateral) was 0.5 *cun*, and for PC-6 (bilateral) the depth was 1 *cun*. For AP treatment D, the sham point was located on the front leg, 3 *cun* away from the middle point of the cubital crease, with 1 *cun* depth of stimulation. Specifically, the locations of the chosen sham points were on the medial side of the front leg bilaterally, since the test AP points, PC-6 and LU-9, were on the medial side (Figure 2). In addition, the sham points were not located on any Regular Channel (LI, LU, SI, HT, TH, and PC). All subjects in AP treatment D used the same sham point anatomic location.

At each visit, the subject was first allowed to acclimate in a quiet room for half an hour. The dog was then carried to the ultrasound room (adjacent to quiet room) to have the baseline blood flow of the femoral artery measured. The dogs were carried rather than allowed to walk, as the act of walking might affect blood flow. After the baseline measurements, the subject was brought to another room where a certified veterinary acupuncturist for dogs and cats performed AP on the subject according to the treatment plan drawn for that visit. After 10 minutes, the needles were removed and the subject was brought back to the ultrasound room again for the post-AP blood flow measurements. The primary investigator (YC), who is an experienced ultrasound clinician (master's degree, radiology), was blinded to the subject's treatment, performed all measures of blood flow and recorded the outcomes.

The study used color ultrasound Doppler image (UCDI), an advanced ultrasound technique, to visualize and assess blood flow in blood vessels. The UCDI includes color Doppler and power Doppler. The color Doppler was used to first localize the femoral artery and then pulsed-wave Doppler was used to measure blood flow. A commercial ultrasound machine[b] with a 10-13 MHz linear transducer was used to generate the outcome data for this study (Figure 3). From each UCDI, the hemodynamic (blood flow) parameters recorded were: vessel diameter (VD), peak systolic velocity (PSV), end-diastolic velocity (EDV), time average maximum velocity (TAMAX), and pulsatility index (PI). Based on these data, the following additional outcome measurements were calculated: (a) resistive index (RI)=(PSV-EDV)/PSV; (b) cross-sectional area (CSA)= $(VD/2)^2 \times \pi$; and (c) blood flow volume (BFV)= CSA×TAMAX. Changes (pre- to post-treatment) of blood flow parameters were compared to investigate the effects of the AP treatment at acupoints PC-6 and/or LU-9.

Figure 3: The image shown is from a study dog, using the ultrasound machine[b] where the transducer was placed on the femoral artery and both color Doppler and pulse Doppler were employed to assess the femoral artery.

Statistical Analysis

The study sought to determine whether AP would affect blood flow in dogs. For example, it was hypothesized that the TAMAX should increase after AP sessions A (LU-9), B (PC-6), and C (LU-9+PC-6). Additionally, A-C treatments should have a greater increase than sessions D (sham) and E (no AP). Since there were 5 AP sessions in this study, all 5 sessions were compared together first: H_0: the mean values of TAMAX increase are the same among the 5 different AP interventions, vs. H_A: the mean values of TAMAX increase are not all the same among the 5 different AP interventions. When a group difference was detected (i.e. H_0 was rejected), individual pairwise comparisons were carried out. To test the hypotheses that compared the mean TAMAX increases among 5 different AP sessions, two-way ANOVA (without interaction term) was applied if the normality assumption held, which was assessed by the Shapiro-Wilk normality test. Otherwise, the nonparametric Friedman test was applied. In cases where the H_0 was rejected by the ANOVA (or Friedman) test, multiple comparisons test (Tukey's HSD test following ANOVA or Conover's test following Friedman test) were applied to determine which two different AP interventions result in a different TAMAX increase. Significance level was set at 0.05 for all tests.

To ensure a 90% power to reject the H_0 (two-way ANOVA comparing 5 different treatments) with a 0.05 significance level, assuming a group difference exists and is equal to the overall sample standard deviation, at least 32 healthy dogs were required. Commercial statistical software was used for all data analysis[c].

RESULTS

A total of 32 clinically healthy dogs were included in this study. All 32 subjects completed 5 rounds (randomized order) of the test AP treatments (including the no-treatment session). Among the 32 subjects, 21 (65.6%) were female (2/21 intact) and 11 (34.4%) were male (7/11 intact). The majority of dogs (28/32=87.5%) were 6 years old or younger (range of 2 months-12 years), with a mean±SD age of 4.1±3.2 years old (median=4.0). For subject's weight, the majority of dogs weighed 10 kg or under (27/32=84.4%) with a mean of 5.8±5.7 kg (median=3.8). All dogs weighed less than 13 kg, except for one subject whose weight was an outlier in the group (32 kg). Subjects in the study were made up of a variety of breeds (Table 1). The most common breeds were Miniature Poodle (34.4%) and Mixed Chinese dog (18.8%).

The pre-AP TAMAX were not significantly different among the 5 AP treatment settings (non-normality data, p=0.058 by Friedman's test). Acupuncture treatments at acupoints LU-9 and PC-6 (A and B, respectively) resulted in an increase in mean TAMAX (A: 1.52±8.40; B: 6.11±8.51), but only treatment B was statistically significant (~23% increase; p<0.001) (Table 2). There was also an increase when subjects received no AP stimulation (1.85±8.94), but was not significant. Acupuncture at both LU-9 and PC-6 (treatment C) yielded a decrease (-1.46±11.57) in TAMAX, which was also observed after the sham AP (treatment D) sessions (-1.12±10.18); neither was significant. Statistically, there existed a significant difference in TAMAX changes among the 5 treatment settings (normality data, p=0.016 by ANOVA test). Based on the post hoc multiple comparisons test (Tukey test), significant treatment differences were found for settings B vs. C (p=0.017) and B vs. D (p=0.026).

The cross-sectional area (CSA) of a vessel is calculated based on its diameter (VD); the wider the vessel (i.e. larger VD), the greater the area (CSA) for blood flow. The pre-AP CSA were not significantly different among the 5 AP treatment settings (non-normality data, p=0.346 by Friedman's test). All 3 AP treatment settings at acupoints (A, B, and C) resulted in an increase in mean CSA, but only treatment B (AP at PC-6) had a statistically significant increase (~18% increase, p=0.005) (Table 3). Both treatment settings D and E reduced the mean CSA, but the change was not statistically significant. The study did not find a significant difference among the 5 treatment settings with respect to CSA changes (non-normality data, p=0.098 by Friedman's test).

Blood flow volume (BFV) is calculated as the product of TAMAX and CSA, which means that the BFV measurement considers both the flow velocity and the thickness of the vessel. There existed a statistically significant difference in pre-AP mean BFV among the 5 AP treatment settings (non-normality data, p=0.025 by Friedman's test), although post hoc multiple comparisons test did not identify a treatment pair with significant difference (B vs. D difference was close; p=0.066). Acupuncture treatments at acupoints LU-9 and PC-6 (A and B, respectively) resulted in the greatest increase in mean BFV, but only treatment B (AP at PC-6) had a statistically significant increase (~56% increase, p<0.001) (Figure 4 and Table 4). Treatments C and E also increased the mean BFV, but changes were not significant. Treatment D reduced the mean BFV, but the change was not statistically significant. There existed a significant difference in BFV changes among the 5 treatments (non-normality data, p=0.025 by Friedman's test). Based on the post hoc multiple comparisons test (Friedman's test), significant treatment differences were found for settings B vs. D (p=0.040).

Table 1: Summary statistics of subject signalment data (n=32)

	Signalment Data
Sex	Female: 21 (65.6%); Male: 11 (34.4%)
Age	2 months – 12 yrs (87.5%≤6 yrs) mean±SD=4.1±3.2 yrs; median=4.0 yrs
Weight	1 kg – 32 kg (84.4%≤10 kg) mean±SD=5.8±5.7 kg; median=3.8 kg
Breed	Poodle (11), Mixed Chinese dog (6), Schnauzer (4), Bedlington Terrier (3), Pomeranian (3), Maltese (2), Bichon Fries (1), Corgi (1), and Labrador (1)

Table 2: Summary statistics (mean±SD) of TAMAX before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	27.97±9.47	29.49±10.30	1.52±8.40	0.313
B. AP at PC-6	26.12±10.33	32.22±11.46	6.11±8.51	<0.001
C. AP at LU-9 & PC-6	31.84±10.44	30.39±10.38	-1.46±11.57	0.482
D. Sham AP	30.11±9.67	28.99±6.79	-1.12±10.18	0.537
E. No AP	30.10±7.64	31.95±11.84	1.85±8.94	0.251

TAMAX=time average maximum velocity; AP=acupuncture; statistically significant value in red

Table 3: Summary statistics (mean±SD) of CSA (cm^2) before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	0.043±0.029	0.044±0.034	0.001±0.016	0.638
B. AP at PC-6	0.040±0.027	0.047±0.028	0.007±0.012	0.005
C. AP at LU-9 & PC-6	0.046±0.035	0.050±0.038	0.004±0.017	0.175
D. Sham AP	0.046±0.025	0.045±0.024	-0.001±0.014	0.677
E. No AP	0.044±0.032	0.043±0.038	-0.002±0.018	0.281

CSA=cross-sectional area; AP=acupuncture; statistically significant value in red

Table 4: Summary statistics (mean±SD) of BFV (mL/min) before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	71.01±50.69	81.41±76.35	10.40±45.24	0.421
B. AP at PC-6	59.54±38.07	93.04±65.72	33.51±47.15	<0.001
C. AP at LU-9 & PC-6	88.51±68.96	89.24±57.33	0.72±53.54	0.940
D. Sham AP	82.25±53.47	78.22±41.84	-4.03±44.05	0.608
E. No AP	78.04±54.75	82.20±81.50	4.16±43.50	0.761

BFV=blood flow volume; AP=acupuncture; statistically significant value in red

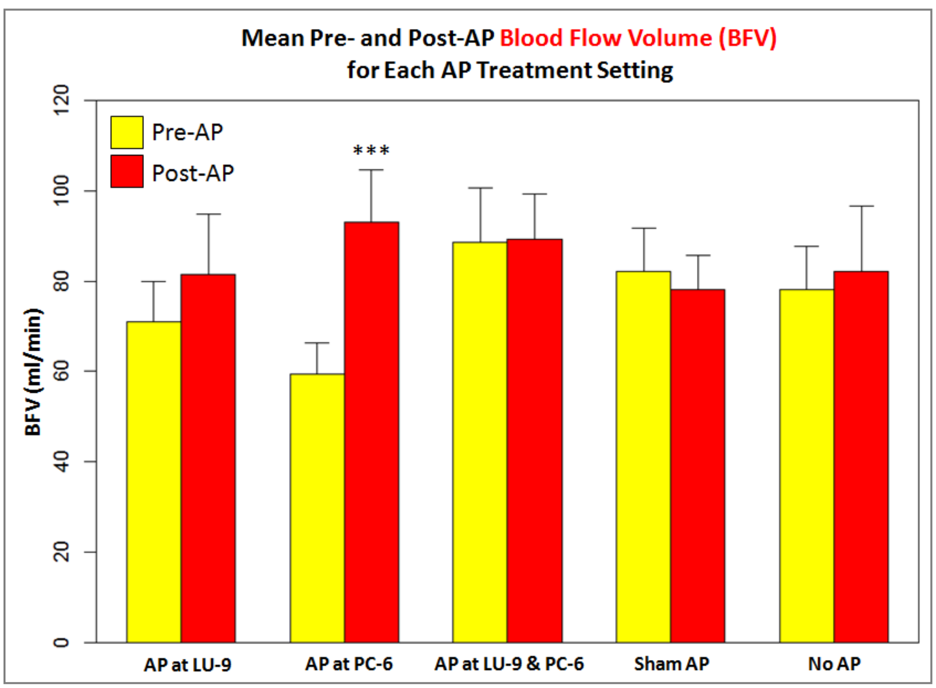

Figure 4: Mean BLV (mL/min) before and after receiving the assigned AP treatment. *** $p<0.001$ compared to the pre-AP temperature (n=32)

Resistive index (RI) is another primary endpoint based on two velocity measures (PSV, EDV), calculated as (PSV-EDV)/PSV. The pre-acupuncture RI were not significantly different among the 5 AP treatments (normality data, p=0.325 by ANOVA test). All AP treatment sessions, as well as the no treatment sessions, resulted in an increase in mean RI, but none of the mean changes were statistically significant (Table 5). Acupuncture treatment session A had the greatest increase among the settings, at least twice any other treatment. The study did not find a significant difference among the 5 treatment settings with respect to RI changes (non-normality data, p=0.590 by Friedman's test).

Further investigations on how treatments differently affected the two velocities (PSV and EDV) associated with RI revealed that treatment B significantly increased both PSV (~18% increase, p=0.001) and EDV (~20% increase, p=0.005), while none of the other treatments had significant effects on either velocity (Tables 6 and 7).

The pre-acupuncture PI were not significantly different among the 5 AP treatment settings (non-normality data, p=0.467 by Friedman's test). Treatments A, C, and E resulted in an increase in mean PI, and the other two treatments (B and D) had decreased mean PI (Table 8). None of the mean changes were statistically significant. The study did not find a statistically significant difference among the 5 treatment groups with respect to PI changes (normality data, p=0.164 by ANOVA test).

Since age can influence vascular health, blood flow values were compared between older (9 through 12-year-old) and younger subjects (2 months through 6-year-old); there were no dogs between 6 and 9 years of age. The majority of dogs were less than 6 years old (87.5%), therefore, percent change to identify trends rather than statistical evaluation was used. Comparison of percent change after AP at PC-6, LU-9, and PC-6+LU-9 for TAMAX, CSA, BFV, RI, PSV, EDV, and PI suggested that younger dogs were more likely to have greater change in blood flow values (increase) when pre- and post-AP parameters were compared at PC-6 and LU-9 (Tables 9 and 10). The combination of the 2 acupoints (PC-6+LU-9), however, demonstrated greater change in blood flow values (increase) for the older dogs (Table 11).

Table 5: Summary statistics (mean±SD) of RI before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	0.776±0.056	0.794±0.058	0.018±0.056	0.079
B. AP at PC-6	0.786±0.058	0.791±0.042	0.005±0.058	0.605
C. AP at LU-9 & PC-6	0.803±0.068	0.812±0.043	0.008±0.061	0.448
D. Sham AP	0.789±0.051	0.792±0.047	0.003±0.055	0.739
E. No AP	0.788±0.062	0.792±0.083	0.004±0.066	0.711

RI=resistive index; AP=acupuncture

Table 6: Summary statistics (mean±SD) of PSV (cm/sec) before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	81.02±26.99	87.83±28.13	6.81±20.5	0.070
B. AP at PC-6	77.14±20.51	91.31±22.61	14.17±21.33	0.001
C. AP at LU-9 & PC-6	92.35±29.06	93.18±25.71	0.831±31.91	0.884
D. Sham AP	91.29±25.72	88.15±18.78	-3.13±26.23	0.963
E. No AP	85.99±23.09	91.88±27.83	5.893±22.10	0.141

PSV=peak systolic velocity; AP=acupuncture; statistically significant value in red

Table 7: Summary statistics (mean±SD) of EDV (cm/sec) before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	17.65±6.04	17.99±7.25	0.34±5.41	0.724
B. AP at PC-6	15.91±4.67	19.12±5.51	3.21±6.05	0.005
C. AP at LU-9 & PC-6	17.73±6.04	17.01±4.87	-0.72±5.85	0.493
D. Sham AP	18.59±5.29	17.81±4.35	-0.78±6.47	0.498
E. No AP	17.41±5.40	18.43±6.04	1.03±5.74	0.320

EDV=end-diastolic velocity; AP=acupuncture; statistically significant value in red

Table 8: Summary statistics (mean±SD) of PI, before and after AP treatment (n=32)

AP Treatment Method	Pre-AP	Post-AP	Change	*p*-value
A. AP at LU-9	2.36±0.54	2.60±0.78	0.24±0.69	0.059
B. AP at PC-6	2.56±0.71	2.43±0.59	-0.13±0.55	0.193
C. AP at LU-9 & PC-6	2.49±0.76	2.67±0.65	0.18±0.74	0.190
D. Sham AP	2.58±0.75	2.54±0.64	-0.05±0.59	0.660
E. No AP	2.39±0.69	2.50±0.86	0.11±0.70	0.402

PI=pulsatility indices; AP=acupuncture

Table 9: Analysis of blood flow parameter change after acupuncture at PC-6, in association with age

	Young Age Group (2 months-6 years) (n=28)		**Old Age Group (9-12 years) (n=4)**	
	Mean Change (SD)	Mean % Change*	Mean Change (SD)	Mean % Change*
TAMAX	6.15 (9.10)	32.6%	5.76 (1.85)	27.0%
CSA	0.01 (0.01)	21.6%	0.01 (0.01)	22.3%
BFV	34.9 (50.2)	69.0%	24.0 (13.0)	59.1%
RI	0.01 (0.06)	1.37%	-0.01 (0.03)	-0.90%
PSV	14.2 (22.3)	23.3%	14.2 (15.0)	20.5%
EDV	3.32 (6.36)	27.3%	2.40 (3.69)	31.4%
PI	-0.14 (0.59)	-1.59%	-0.09 (0.30)	-3.92%

*Red font- greater increased blood flow/or less decreased blood flow parameter compared to other age group

Table 10: Analysis of blood flow parameter change after acupuncture at LU-9, in association with age

	Young Age Group (2 months-6 years) (n=28)		**Old Age Group (9-12 years) (n=4)**	
	Mean Change (SD)	Mean % Change*	Mean Change (SD)	Mean % Change*
TAMAX	1.91 (8.56)	11.0%	-1.21 (7.63)	-2.59%
CSA	0.003 (0.01)	10.9%	-0.008 (0.02)	-14.7%
BFV	15.1 (61.6)	28.8%	-22.2 (24.0)	-20.3%
RI	0.01 (0.06)	2.13%	0.04 (0.02)	5.56%
PSV	7.20 (21.8)	13.3%	4.07 (7.93)	6.73%
EDV	0.69 (5.60)	6.51%	-2.10 (3.30)	-10.3%
PI	0.20 (0.70)	10.4%	0.50 (0.61)	23.1%

*Red font- greater increased blood flow/or less decreased blood flow parameter compared to other age group

Table 11: Analysis of blood flow parameter change after acupuncture at LU-9+PC-6, in association with age

	Young Age Group (2 months-6 years) (n=28)		**Old Age Group (9-12 years) (n=4)**	
	Mean Change (SD)	Mean % Change*	Mean Change (SD)	Mean % Change*
TAMAX	-3.68 (9.95)	-6.51%	14.1 (11.0)	49.8%
CSA	0.003 (0.02)	21.2%	0.01 (0.02)	37.2%
BFV	-9.79 (44.2)	12.2%	74.3 (61.8)	134.4%
RI	0.01 (0.05)	1.68%	-0.01 (0.11)	0.61%
PSV	-3.18 (30.7)	1.47%	28.9 (29.1)	39.5%
EDV	-1.35 (5.91)	-0.78%	3.73 (3.00)	29.5%
PI	0.26 (0.69)	15.9%	-0.40 (0.92)	-8.32%

*Red font- greater increased blood flow/or less decreased blood flow parameter compared to other age group

DISCUSSION

This repeated measure randomized controlled study with blinded assessments sought to determine the effect of AP stimulation at acupoints LU-9 and/or PC-6 on blood flow in dogs. The main findings from the blood flow data collected through UCDI technique were: a) significant differences were found between PC-6 AP and sham or no AP treatments for TAMAX, CSA, BFV, PSV and EDV; b) none of the other AP treatments (A, C), including sham (D) and no treatment (E) had significant effects on blood flow parameters; and c) none of the AP treatments had significant effects on RI and PI. These findings partially satisfied the study hypothesis with significant increases for PC-6 but not LU-9 or PC-6+LU-9.

Study findings shed light on the specific impact of PC-6 AP stimulation on blood flow parameters in dogs, offering valuable insights for further exploration and potential clinical applications. In comparing the results among different AP treatments, it is crucial to consider the uniqueness of PC-6 stimulation and its distinct effects on various blood flow parameters. When compared to stimulation of a sham point, AP stimulation at PC-6 resulted in significant changes (increases) in TAMAX (23% vs. 4% from sham AP), CSA (18% vs. 2% from sham AP), BFV (56% vs. 5% from sham AP), PSV (18% vs. 3% from sham AP), and EDV (20% vs. 4% from sham AP). While LU-9 is traditionally considered the "Influential point for pulse" in TCM and TCVM, the scientific evidence reported herein challenges these specific claims, as comparison between PC-6 and LU-9 outcomes revealed that PC-6 exerted a stronger effect on blood flow under the experimental conditions of this study.

Notably, none of the AP treatments demonstrated significant effects on the resistant indices (PI and RI), both calculated based on PSV and EDV. This might be due to factors such as vasodilation, autoregulation, or compensatory mechanisms that maintained the overall balance in blood flow dynamics. Furthermore, this could suggest that the AP treatment did not significantly alter the resistance to blood flow or the compliance of the arterial system. Alternatively, it might indicate that any changes in peak systolic and end diastolic velocities were proportionate, maintaining the overall pulsatility, suggesting that the pulsatility or the variation in blood flow throughout the cardiac cycle has remained relatively stable.

It is also important to note that the present study utilized UCDI to measure changes in blood flow following AP treatment. This non-invasive method allows for accurate assessment, enhancing the reliability of the findings. As this was the first reported clinical study using UCDI to assess canine blood flow effects from AP treatments, after delving into the implications of the distinctive impact of PC-6 stimulation on blood flow parameters, further discussions and comparisons with relevant clinical studies should be expected and welcomed.

While the study on AP point stimulation to investigate its effects on blood flow parameters in dogs has provided valuable insights, it is essential to acknowledge certain limitations and unexpected outcomes that may guide future research endeavors. Firstly, the study's sample size, comprising 32 clinically healthy dogs, although carefully selected, might pose limitations in terms of generalizability to broader canine populations or specific clinical conditions. A larger and more diverse sample would allow more even distribution of physical variations in dog populations and could enhance the external validity of study findings. Additionally, the exclusive focus on clinically healthy dogs might limit the applicability of the results to dogs with underlying health issues. Future studies should explore the impact of AP point stimulation on blood flow in dogs with specific medical conditions to broaden clinical relevance. Furthermore, the study's design, which involved five rounds of randomized AP treatments with at least 1 week washout period between treatments, might have introduced a learning effect or habituation in the dogs, potentially influencing their responses over subsequent sessions. A more varied and extended treatment schedule or exploring alternative randomization methods could help mitigate this potential carryover effect.

Vascular health and blood flow can be influenced by age, differences in anxiety levels, activity or environmental factors. Reduction of variables such as environment, anxiety and activity were controlled by using dogs familiar with the environment at the pet hospital and carrying dogs between rooms to eliminate walking, with the exception of 1 dog which was too heavy to carry. All dogs in the study appeared relaxed, and quiet rooms located adjacent to treatment areas were used, so it is thought that these variables did not have a significant influence on study results.

Although most dogs in the study were equal to or younger than 6 years old (87.5%), blood flow values were compared between older and younger subjects to investigate this variable. Comparison of percent change after AP at PC-6 and LU-9 for TAMAX, CSA, BFV, RI, PSV, EDV, PI suggested that younger dogs were more likely to have greater change in blood flow values (increase) when pre- and post-acupuncture parameters were compared (Tables 9, 10). In contrast, when both acupoints were used together (i.e. PC-6+LU-9), a greater effect on blood flow (increase) was seen in the older dogs (Table 11). This is an interesting finding and would be important to investigate in a larger statistically meaningful group in future studies. The effect on findings in this study, with most dogs being young, is considered small. It suggests, however, that age matched animals should be used for future studies.

Unexpected outcomes, such as the observed differences in the effects of LU-9 and PC-6 on blood flow parameters, warrant further investigation. The study revealed that the stimulation of PC-6 resulted in a discernible increase in blood flow, aligning with the traditional understanding of its influence on cardiovascular dynamics. In contrast, the lack of a significant increase in blood flow measures following the stimulation of LU-9 raises intriguing questions about the traditional idea of LU-9, which is the Influential Point for the pulse and vessels. The combined treatment (LU-9+PC-6), explored with the anticipation of a potential synergistic effect, did not lead to a significant increase in blood flow measures under the experimental conditions of this study. While the precise reasons for these unexpected outcomes remain elusive, it prompts consideration of intricate interactions between these acupuncture points and the need for further exploration into optimal stimulation parameters, and the underlying mechanisms governing their effects. These unexpected findings highlight the complexity of acupuncture interventions and warrant additional investigation to unravel the nuances influencing blood flow regulation.

In conclusion, based on color Doppler imaging, the findings from this study suggest that AP treatment can increase blood flow, especially with respect to the flow velocity and volume. The ability to detect blood flow differences secondary to acupuncture in healthy dogs opens the door for studies that focus on dogs with specific medical conditions, extend to different species, or investigate the sustained effects of AP on blood flow over extended periods. By pursuing these future research directions, the field of acupuncture in veterinary medicine can advance its scientific foundation, optimize treatment protocols, and contribute to the broader understanding of complementary and alternative therapies for animal health and well-being.

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FOOTNOTES

[a] Acupuncture needles (sterile, single-use), Beijing Hanyi Medical Instruments Co., LTD, Beijing, China

[b] G30VET, Shenzhen Emperor Electronic Technology Co. Ltd, Shenzhen, China

[c] R version 4.4.1. The R Foundation for Statistical Computing, Vienna Austria; http://www.R-project.org

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