

Clinical Studies

Pilot Study to Assess the Effect of *An-shen* Aqua-acupuncture on Heart Rate in Healthy Dogs: A Randomized, Cross-over Trial

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ABSTRACT

The objective of this randomized, crossover, pilot study was to assess the effect of aqua-acupuncture (aqua-AP) at *An-shen* acupoint on heart rate (HR) in healthy dogs. Thirteen dogs were randomized to receive aqua-AP at *An-shen* acupoint using solutions of normal saline, Traumeel®, and cyanocobalamin (vitamin B₁₂) with a minimum of one-week washout period between crossovers. An ambulatory electrocardiography (Holter) was recorded for 24 hours before and after each aqua-AP to assess the changes in 24-hour mean HR, maximum HR, and minimum HR. No dogs experienced any adverse effects from aqua-AP treatment. After aqua-AP, 91% of dogs with vitamin B₁₂, 60% with Traumeel®, and 42% with normal saline experienced a decrease in 24-hour mean HR. The mean (with 95% confidence interval) decreases in 24-hour mean HR (bpm) from baseline was 4.18 (1.8 to 6.5) with vitamin B₁₂, 3.9 (-3.7 to 3.1) with Traumeel®, and -0.25 (-7.7 to -1.1) with normal saline. The changes in maximum and minimum HR were variable with wider confidence estimates around the mean change in the respective HR's for any aqua-AP solution. The results of this pilot study show that aqua-AP with vitamin B₁₂ provided the greatest decrease in 24-hour mean HR in healthy dogs. Further confirmatory studies are warranted to assess the effect of aqua-AP on HR in clinical conditions that warrant HR reduction.

Keywords: *An-shen*, aqua-acupuncture, arrhythmias, canine, heart rate, Holter

ABBREVIATIONS: aqua-AP: aqua-acupuncture; ECG: electrocardiography; Holter: 24-hour ambulatory ECG; HR: heart rate; vitamin B₁₂: cyanocobalamin

Acupuncture is gaining increasing acceptance as an alternative or complementary therapy for treating various conditions in veterinary patients.¹ Aqua-acupuncture (aqua-AP) is a specific method of acupuncture where a liquid substance is injected at the acupoint, delivering prolonged mechanical and neuronal stimulation.^{2,3} Various substances, including normal saline, vitamin B₁₂, homeopathic compounds, drugs, or herbal medications, are currently employed for aqua-AP in clinical practice. Notably, diluted, or undiluted vitamin B₁₂ is a commonly used preparation for aqua-AP. Another compound, Traumeel®^a is a fixed combination of biological and mineral extracts renowned for its anti-inflammatory properties in humans and has gained popularity among veterinary acupuncturists.⁴ Typically, aqua-AP is recommended for patients who cannot tolerate the extended duration of traditional dry needle acupuncture at acupoints, offering a viable option for veterinary patients often stressed in a clinical environment.³ Few studies have assessed the effectiveness of aqua-AP at various acupoints in small animals.⁴⁻⁶ A recent study demonstrated that incorporating aqua-AP with vitamin B₁₂

at the Governing Vessel acupoint (*Chang-qiang*, GV-1) into conventional treatment for acute diarrhea led to shorter duration of clinical symptoms.⁵

In human patients, acupuncture has been shown to consistently decrease resting HR and improve heart rate variability.⁶⁻⁸ Theoretically, the reduction in HR is achieved by modulation of the autonomous nervous system by enhancing vagal activity and by suppressing sympathetic activation.⁷⁻⁹ These findings were also supported by experimental studies in rats, where acupuncture stimulation of lower limbs, resulted in reduced blood pressure and HR.^{10,11} Interestingly, the magnitude of the sympathetic inhibitory response noted appears to be directly related to the basal activity of the sympathetic nervous system.^{12,13}

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Studies evaluating the impact of acupuncture on HR are limited in clinical veterinary patients. One study evaluated the use of bilateral aqua-AP at *An-shen* acupoint on stress behaviors associated with veterinary clinic visits in dogs.⁴ *An-shen* (pacify *Shen*), a classical acupoint, is not linked to any specific Meridian or the 365 transpositional acupoints. It is located on the posterior aspect of the ear, positioned halfway between *Yi-feng* (TH-17) and *Feng-chi* (GB-20).¹⁴ The anatomic location of this acupoint contains two cervical nerves and four cranial nerves coursing through the area.¹⁴ Functioning as a soothing point, *An-shen* acupoint is commonly used for relieving conditions such as anxiety, fear, grief, and timidity in both dogs and cats.¹ The above-mentioned study showed that dogs receiving vitamin B₁₂ aqua-AP had significant improvement in behavior scores as compared to dogs receiving sham aqua-AP at a non-acupuncture point.⁴ Interestingly this study did not show any significant differences in heart rate (HR) reduction between sham aqua-AP and vitamin B₁₂ aqua-AP at *An-shen*.⁴ A similar study, compared the use of aqua-AP with vitamin B₁₂ at *An-shen* and GB-21 to therapy with trazodone on stress behaviors in dogs during clinic visits.⁶ While there was a significant reduction in HR in all aqua-AP groups 30 minutes after examination, the degree of HR reduction was not significantly different between different aqua-AP groups or with trazodone therapy.⁶ In both studies, HR was obtained by auscultation at baseline, and at 30 minutes post aqua-AP.^{4,6} One possible explanation for the lack of significant findings is that the HR naturally decreases in dogs after the initial excitement of a clinic visit in all groups and to capture the true effect of aqua-AP on HR, a longer duration HR assessment may be necessary. Long-term ECG recordings such as ambulatory ECG recordings (Holter) provide a better assessment of long-term changes in HR including the maximum and minimum HR fluctuations in a dog's natural home environment.¹⁵

The objective of this pilot study was to assess the effect of aqua-AP using normal saline, vitamin B₁₂, and Traumeel® at *An-shen* acupoint on 24-hour mean HR, minimum HR, and maximum HR in healthy dogs as assessed by 24-hour Holter recordings.

MATERIALS AND METHODS

Thirteen healthy dogs owned by students and employees at the Veterinary Teaching Hospital, Michigan State University were prospectively evaluated for recruitment into the study. Each dog received a thorough history and physical exam by a veterinarian as part of the screening evaluation. Dogs with known heart disease, abnormal cardiac auscultatory findings, or dogs that were receiving any medications that could affect HR as determined by the evaluation of history and physical exam findings, were excluded from the study. An informed consent was obtained from the owners and the study protocol was approved by the Institutional Animal Care & Use Committee at Michigan State University.

On Day 1 of the trial, each enrolled dog was fitted with a Holter monitor^b with continuous 48-hour recording capacity to obtain baseline 24-hour ECG data (pre-Holter), (Figure 1). Holter monitors were applied to the dogs using a previously described technique.¹⁶ Briefly, two areas (approximately 4 inch) of fur were shaved on either side of the chest behind the axilla. A four channel Holter recorder was fitted by applying two electrode patches on each side. The Holter recorder and the electrode patches were finally secured using a fitting Holter vest.

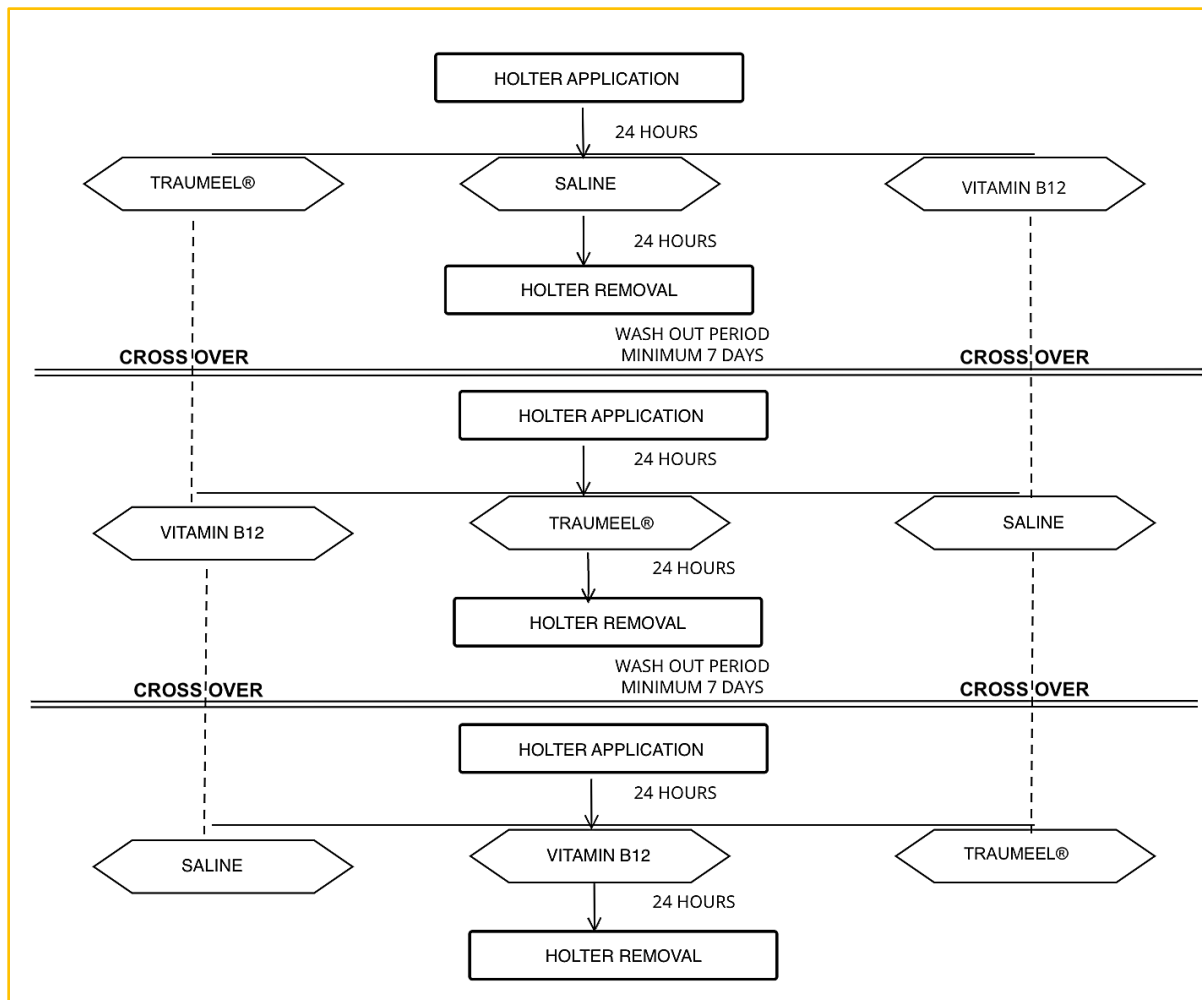
After 24 hours of wearing the Holter (Day 2), each study dog underwent aqua-AP therapy with one of the following solutions: normal saline^c, vitamin B₁₂ (1000 mcg/mL)^d, or Traumeel®^a. The assignment of the aquapuncture solution was randomized using a random table with two dogs completing a specific treatment order for each group (Table 1). Each dog received their assigned aqua-AP with 1 mL of the undiluted solution using a 3 mL syringe, 22 G hypodermic needles^e, bilaterally at *An-shen* acupoint. Aquapuncture was performed by two certified veterinary acupuncturists.

After aqua-AP, Holter recording was continued for another 24 hours (post-Holter) to assess treatment response. After initial Holter placement (Day 1) and aqua-AP (Day 2), the dogs were sent home to interact with their customary home environments. The owners were encouraged to standardize activities at home during this time to the best of their abilities. They were also provided a Holter diary to record any events that could alter the HR of the dog. A 20-minute duration of exercise such as walking was advised for each dog at the same time each day. No other changes from the dog's normal routine in diet, exercise, or environmental stimuli were requested. The owner returned for the removal of the Holter monitor on Day 3. After a washout period of minimally seven days, each dog completed the other arms of the study protocol with other solutions by undergoing similar pre- and post-Holter recordings as noted above (Figure 1).

The Holter recordings were analyzed using automated computer software^f with analyses verified by a board-certified veterinary cardiologist. This verification process involved the detection of arrhythmias that were identified as normal complexes by the automated system and vice versa, assessment for normal triggering, removal of artifacts, and manual verification of arrhythmic events. For every 24 hours of the Holter recording, the mean heart rate per 24-hour period, maximum heart rate, and minimum heart rate was calculated. The latter two were calculated from rolling one-minute averages. If there were arrhythmias, the frequency of each arrhythmia was tabulated for each dog. Dogs that had more than 5% artifacts on their 24-hour Holter recording or had more than 5% ventricular or supraventricular arrhythmia burden were removed from the study. Normality was tested using the Anderson-Darling test. Normally distributed variables were depicted in mean and standard deviation. Non-normally distributed variables were depicted in median and range. A commercial statistical software was used to calculate central tendencies and two-tailed 95% confidence interval estimates^g.

Table 1: Randomization and group assignment table for the study

Groups	Injection 1	Injection 2	Injection 3
1	Vitamin B ₁₂	Normal saline	Traumeel®
2	Vitamin B ₁₂	Traumeel®	Normal saline
3	Normal saline	Vitamin B ₁₂	Traumeel®
4	Normal saline	Traumeel®	Vitamin B ₁₂
5	Traumeel®	Vitamin B ₁₂	Normal saline
6	Traumeel®	Normal saline	Vitamin B ₁₂

**Figure 1:** Flow chart of study protocol to assess the effect of *An-shen* aqua-acupuncture on heart rate in healthy dogs

RESULTS

One dog did not complete the study due to the dog chewing the Holter equipment after administration of the first aqua-AP solution. This dog was excluded from further analysis. Twelve dogs completed the study with a mean body weight of 24.5 kgs (standard deviation = 8.9 kgs, range = 11 kg to 45 kg). Breeds of dogs included Labrador Retriever (3), German Shephard (1), Siberian Husky (1), North American Saluki (1), English Springer Spaniel (1), American Staffordshire Terrier (1), and mixed breed (4). Four dogs were spayed females, and eight dogs were

neutered males. The mean age of study dogs was 5.6 years (standard deviation = 2.5 years, range = 1 to 10 years). All twelve dogs completed aqua-AP with saline solution. Two dogs did not complete aqua-AP treatment with the Traumeel® solution and one dog did not complete aqua-AP treatment with the vitamin B₁₂ solution. None of the dogs had more than 5% artifacts or arrhythmia burden on any of the Holter recordings. No study dogs experienced any adverse effects to aqua-AP or Holter monitor application.

Table 2: Difference in HR variables between pre- and post-aqua-AP therapy in healthy dogs

Groups	24-hour Mean HR (bpm) Mean (std)		Maximum HR (bpm) Mean (std)		Minimum HR (bpm) Mean (std)	
	Pre-Holter	Post-Holter	Pre-Holter	Post-Holter	Pre-Holter	Post-Holter
Vitamin B ₁₂ (n=11)	72.9 (6.5)	68.2 (6.1)	43.8 (5.3)	43.3 (3.9)	183.6 (13.9)	180 (17.9)
Saline (n=12)	69.7 (7.9)	70 (9.1)	42 (5.4)	40.3 (7.5)	183.5 (19.5)	182.1 (21.9)
(Traumeel® n=10)	72.3 (6.0)	68.4 (7.5)	42.2 (10.3)	39.3 (9.1)	187.6 (13.3)	183 (17.7)
Groups	24-hour Mean HR (bpm) Median (range)		Maximum HR (bpm) Median (range)		Minimum HR (bpm) Median (range)	
	Pre-Holter	Post-Holter	Pre-Holter	Post-Holter	Pre-Holter	Post-Holter
Vitamin B ₁₂ (n=11)	71 (62-82)	71 (59-76)	44 (32-52)	44 (36-48)	184 (161-202)	183 (144-204)
Saline (n=12)	68.6 (58-80)	69 (56-84)	43 (32-51)	42 (24-54)	182 (159-216)	179 (152-210)
Traumeel® (n=10)	72.5 (62-80)	68 (60-83)	45 (28-53)	41 (24-53)	186.5 (167-214)	189.5 (152-206)

HR: heart rate; std: standard deviation

Table 3: Difference in HR variables and percentage of healthy dogs that had decrease in HR parameters between pre- and post-aqua-AP therapy

Treatment	24-hour Mean HR (bpm)		Maximum HR (bpm)		Minimum HR (bpm)	
	Mean diff (95% CI)	% dogs	Mean diff (95% CI)	% dogs	Mean diff (95% CI)	% dogs
Vitamin B ₁₂ (n=11)	4.18 (1.8 to 6.5)	91	3.5 (-7 to 14)	63	1.6 (-3.4 to 4.3)	63
Saline (n=12)	-0.25 (-7.7 to -1.1)	42	1.3 (-17 to 13)	66	1.6 (-4.1 to 6.5)	66
(Traumeel® n=10)	3.9 (-3.7 to 3.1)	60	4.6 (-14 to 17)	50	2.9 (-3.1 to 8.0)	80

HR: heart rate; mean diff: difference in HR between pre- and post-Holter for the parameters of interest; 95% CI: 95% confidence intervals

The 24-hour mean HR, maximum HR, and minimum HR on pre- and post-Holter recordings after aqua-AP are depicted in Table 2. Briefly, 10 out of 11 dogs (91%) in the vitamin B₁₂ group, 6 out of 10 (60%) in the Traumeel® group, and 5 out of 12 (42%) in the normal saline group, experienced a decrease in 24-hour mean HR post-aqua-AP. The mean (with 95% confidence interval) decreases in 24-hour mean HR (bpm) from baseline was 4.18 (1.8 to 6.5) with vitamin B₁₂, 3.9 (-3.7 to 3.1) with Traumeel®, and -0.25 (-7.7 to -1.1) with normal saline. The mean (with 95% confidence interval) decreases in maximum HR (bpm) from baseline was 4.6 (-14 to 17) with Traumeel®, 3.5 (-7 to 14) with vitamin B₁₂, and 1.3 (-17 to 13) with normal saline. The mean (with 95% confidence interval) decrease in minimum HR (bpm) from baseline was 2.9 (-3.1 to 8.0) with Traumeel®, 1.6 (-3.4 to 4.3) with vitamin B₁₂, and 1.6 (-4.1 to 6.5) with normal saline (Table 3).

DISCUSSION

The results of this pilot study demonstrated that both aqua-AP with vitamin B₁₂ and Traumeel® at *An-shen* acupoint reduced 24-hour mean HR in healthy dogs. Of note, the degree of decrease in HR and the 95% CI around the mean decrease in HR appear to be better with vitamin B₁₂ as compared to Traumeel®. Additionally, aqua-AP with normal saline did not produce an appreciable change in the 24-hour mean HR.

This finding is similar to studies in healthy humans⁷ where a single session of dry needle acupuncture at various acupoints has been shown to reduce HR.¹⁷ The degree of HR reduction noted in healthy humans with acupuncture is very similar to the HR reduction noted in our study.¹⁷ To our knowledge, there are no human or veterinary studies that directly evaluated the effect of acupuncture on HR using 24-Holter recordings. Holter recordings provide a better assessment of a dog's treatment response and are less influenced by short-term fluctuations in HR by the excitement of clinic visits.¹⁵ Two previous veterinary studies in dogs showed HR reduction with aqua-AP within 30 minutes of examination but failed to show any statistically significant differences in the degree of reduction in HR when compared to sham aqua-AP or control groups.^{4,6} It is possible that shorter duration HR assessment in the above-mentioned studies did not allow enough time to assess the true benefit of aqua-AP on HR. By performing longer-term HR evaluation using Holter recordings in our study the true effect of aqua-AP on HR was captured.

In aqua-AP, the introduction of a liquid substance induces alterations in the local spatial configuration.² The changes in spatial arrangement and the properties of the liquid substance itself contribute to the stimulation of the acupoint, resulting in activation of the neuronal system.² In one study the degree of neuronal activation was assessed after aqua-AP using different substances, and found that the degree of activation was not significantly different

between different substances except for bee venom aqua-AP.² This study, however, did not assess the longevity of effects after a single aqua-AP which may be different between different substances used in aqua-AP.

In our study, aqua-AP with normal saline did not produce a significant reduction in HR as compared to aqua-AP with B₁₂. It is possible that even if there was an initial effect of normal saline aqua-AP on HR, the HR-reducing effect did not last long enough to produce a detectable difference in HR on Holter recording. Similarly, the shorter duration of effect as compared to vitamin B₁₂ may also explain the lack of significant effect on HR noted with aqua-AP. The response and the duration of the effect received from aqua-AP, therefore, may also depend on the substance used. Interestingly the effect of aqua-AP on maximum HR or minimum HR was quite variable in this study. These high and low HR patterns can be significantly affected by activity levels of dogs at home and can be influenced by factors such as animal owner interactions and other extraneous factors.

Several human studies have evaluated the use of acupuncture in the treatment of various cardiac arrhythmias. In particular, a meta-analysis of various acupuncture studies has shown that acupuncture is safe and effective in treating various supraventricular tachycardias.¹⁸ More specifically, acupuncture is effective in cardioversion and controlling HR in human patients with atrial fibrillation.¹⁹ The HR-reducing effect of aqua-AP at *An-shen* acupoint is encouraging and needs to be further confirmed in larger trials and later in dogs with various supraventricular tachyarrhythmias that might benefit from modulation of HR.

Our study has several limitations. The current study did not have a true sham aqua-AP group or a control Holter group. The concerns of having a sham acupuncture group in acupuncture trials have been discussed and discouraged when designing acupuncture clinical trials.²⁰ Without a control Holter group, one may argue that the decrease in HR between pre- and post-aqua-AP Holter recordings noted in this study may be due to the acclimatization of dogs to wearing the Holter monitor rather than the true effect of aqua-AP. There were, however, no major changes to HR after aqua-AP with normal saline. If there was a significant effect of Holter acclimatization on the HR, we would have expected to see a significant decrease in HR with all solutions including normal saline aqua-AP. In future confirmatory trials, having a control group to study the effect of acclimatization to Holter monitoring on HR may be reasonable. Despite our best efforts, the type of physical activity or environmental exposure to external stimulus while wearing the Holter monitor was not standardized in all dogs. Such variation in activity levels or stressors could have influenced the HR patterns noted on the Holter monitor. While, each owner was asked to maintain a similar type of activity levels, before and after aqua-AP therapy while wearing the Holter monitor, the activity level and owner-animal interactions should be further standardized in any future confirmatory trials.

The sex of the dogs and influence of sex on response to aqua-AP was not considered in randomization of

participant allocation to groups in this study. Sex, however, is not known to influence HR parameters on Holter monitoring in altered dogs and as such not expected to have played a major role in our results.²¹ Additionally, concurrent analgesic effects of Traumeel® and its potential effect on HR by pain control in dogs with subclinical pain should be considered.²² Most dogs in this study, however, were young to middle aged dogs (only two dogs older > 8 years) and had no reported chronic history of pain. Additionally, we used a single dose of 1 mL for aqua-AP in all dogs despite variation in body weight. This single dosing may be a limitation if there are any actual dose related responses to the effect of aqua-AP on HR. While the exact dosing protocol for aqua-AP remains unknown in veterinary patients, it may be reasonable to consider a graded dosing protocol based on body weight in future trials. Finally, the specific duration of action of Traumeel® aqua-AP is unknown and it is possible that the washout period used in this study was not adequate. Due to sample size and drop out of two dogs after Traumeel® in this study, such carry over effects beyond washout period could not be properly assessed. In future trials, these limitations should be considered in study design and patient recruitment.

In summary, the findings of this study demonstrate that aqua-AP at *An-shen* with vitamin B₁₂ reduces HR in healthy dogs. Further confirmatory trials are warranted in assessing the effect of aqua-AP at *An-shen* and its potential to treat various cardiac arrhythmias in veterinary patients.

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Declaration of Interest and Funding

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FOOTNOTES

- ^a Traumeel®, Biologische Heilmittel Heel GmbH, Baden-Baden, Germany
- ^b Lifecard CF, Spacelabs Healthcare, Snoqualmie, WA, USA
- ^c Normal saline, Henry Schein Medical, Alpharetta, GA, USA
- ^d Vitamin B₁₂, MWI Animal Health, Boise, ID, USA
- ^e Terumo syringes and needles, MWI Animal Health, Boise, ID, USA
- ^f Pathfinder SL, Spacelabs Healthcare, Snoqualmie, WA, USA
- ^g R version 3.5.2. The R Foundation for Statistical Computing, Vienna, Austria; <http://www.R-project.org>

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