Bone Markers in Charcot Neuroarthropathy

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Research

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Abstract

Background

There have been multiple studies evaluating the effectiveness of pharmaceuticals in the treatment of acute Charcot neuroarthropathy (CNA). The effectiveness of most pharmaceuticals historically has been based on the levels of certain biomarkers of bone turnover. Previous research has shown bone turnover markers are increased in acute phase of CNA. However, biomarkers activity in chronic phase of CNA and its utility for monitoring treatment response remains to be established. In this article, we evaluated the relationship of such biomarkers to disease severity. We hypothesized that biomarkers such as BSAP and DPD:CRT levels will be directly proportional to disease severity i.e. increased in acute CNA and decreased in chronic CNA. Hence pharmacologic treatment in acute CNA with reference to levels of such biomarkers potentially could be beneficial in clinical settings.

Methods

We retrospectively reviewed 41 patients diagnosed with acute and chronic CNA in our Charcot clinic. Disease severity was determined via pedal temperature difference between affected and unaffected limbs using an OMEGA Surface Temperature Scanner in conjugation with radiographic films and clinical presentation. Temperature of 2 degrees Celsius or greater is considered acute CNA, temperature less than 2 degree Celsius is considered chronic CNA. Urine and serum blood samples were collected at baseline to evaluate the levels of BSAP and DPD:CRT via immunoassays. Statistical analysis was performed via separate Mann Whitney rank sums tests to determine between-group differences in BSAP and DPD:CRT in acute versus chronic CNA.

Results

Bone formation marker BSAP ($p = 0.46$) and bone resorption marker DPD:CRT ($p = 0.92$) were not significantly different in acute versus chronic CNA. In acute CNA patients ($n = 31$) median BSAP was 11.9 (range 3.8–41.3) versus 15.2 (range 4.4–40.7) in chronic CNA patients. In acute CNA patients, median DPD:CRT was 7.8 (range 3.1–38.4) versus 8.8 (range 3.3–18.6) in chronic CNA patients.

Conclusion

The lack of significant between-group differences in BSAP and DPD:CRT calls into question their reliability in monitoring pharmaceuticals effectiveness in acute versus chronic CNA.

1. Introduction

Neuropathic osteoarthropathy, often referred to as Charcot neuroarthropathy (CNA) after the French neurologist Jean Martin Charcot, is a severely debilitating condition that was first described by Musgrave as an arthritis associated with venereal disease in 1703 [1]. Charcot himself described the neuropathy as an essential component to this disease in 1868 [2]. Jordan, in 1936, associated this condition with Diabetes Mellitus [3]. Diabetes Mellitus is now thought to be the most common cause for the development of CNA [4]. The basic physiopathologic mechanism of CNA is poorly understood although repetitive trauma and autonomic nervous dysfunction are probably implicated (the neurotraumatic and neurovascular theories). CNA can present either in acute or chronic stage, and its features vary according to the stage of presentation. Acute CNA presents with a red and swollen foot, which is warmer than the contralateral normal foot. Resolution of inflammation and establishment of residual deformity characterize chronic CNA. The hallmark of this condition is midfoot collapse, described as a “rocker-bottom” foot although the condition appears in other joints and with other presentations. As the deformity progresses, it can often lead to areas of increased plantar foot pressure [5]. Areas of increased pressure predispose a patient to ulceration, particularly if there is accompanying neuropathy. Ulcerations frequently lead to infections, which commonly lead to lower extremity amputation, sepsis and even death.

Patients with acute CNA often presents to clinic with unresolved edema and erythema of the affected limb. If only one limb is affected, it will be warmer than the other limb by at least 2 degrees Celsius [2]. Despite the fact that this disease is most common in neuropathic patients, pain at presentation is not uncommon. The clinician might also observe hypermobility and instability of joints on the affected side [6]. Patients with CNA have been shown to have a greater incidence of osteopenia in the lower extremity as compared to non-CNA neuropathic patients [7]. This finding adds understanding to the pathologic process of fractures and fragmentation of the foot in patients with CNA. Hyperactive, or unopposed osteoclasts are thought to be accountable in part for bone demineralization in the acute stage of CNA [7]. Since infection can present with many of these same symptoms, the diagnosis of acute CNA can be challenging and often requires further investigation via imaging.

There are various imaging modalities that aid in the diagnosis of CNA such as plain radiography, computed tomography, magnetic resonance imaging and three phase bone scans. Newer imaging such as fluorodeoxyglucose-positron emission tomography (FDG- PET) and single-photon emission computed tomography/computed tomography (SPECT/CT), which are showing accuracy in the differentiation of Charcot from osteomyelitis in the range of 90–100% [8]. The gold standard for diagnosing Charcot remains pathological evidence of bone shards in the synovium of the affected joint [2]. However, this is not always practical, and clinical suspicion together with MRI or bone scan is usually adequate. More important is the early recognition of the disease, Armstrong et al. highlighted the need for higher clinical suspicion in the insensate foot, and promote early offloading interventions to prevent the profoundly debilitating sequelae of delayed diagnosis [9].

Once diagnosed, CNA will progress through multiple stages. Eichenholz introduced the classic staging system of Charcot in 1966. This staging system was based on radiographic changes, and the stages are as follows: Stage I: developmental, Stage II: coalescence, and Stage III: remodeling [10]. More recently a

stage 0 has been added to this system. The Stage 0 patient has clinical signs of edema, erythema, and warmth, and they may or may not exhibit radiographic changes [6]. Although other staging systems are mentioned, the current authors feel the most pertinent is the system presented by Armstrong and associates. Their algorithm demonstrated two stages, acute and post-acute (chronic), based on radiographic, temperature differentiation, and clinical presentation [9]. Pedal temperature comparisons from affected limb to the contralateral side are considered paramount in the current authors opinion and have been shown to correlate with disease activity [11]. An acute process should be suspected if the temperature of the affected foot is 2 degree Celsius or greater than the contralateral unaffected foot. This is usually measured using an infrared thermometer [18].

Current treatment regimens of CNA consist of immobilization and offloading to prevent deformity and ulceration, surgical planning of bony prominences, or total surgical reconstruction [3, 10]. Effective medical therapy is still in investigational stages and has mostly been based on various biochemical markers utilized to gauge osteoclastic activity, and thereby gauge the progression of CNA [2, 12–15]. Among these markers are deoxypyridinoline crosslinks (DPD), a bone resorptive marker, and bone specific alkaline phosphatase (BSAP) a bone formation marker. Deoxypyridinoline crosslinks provide stabilization for Type I collagen, which is most commonly found in bone. When bone turnover is high these crosslinks spill over into the urine. BSAP is an enzyme produced by activated osteoblasts that appears to have a role in calcium hydroxyapatite deposition on bone. Other bone markers have been utilized to monitor acute CNA, including urinary N-terminal peptide (NTX), serum carboxy-terminal telopeptide domain of type 1 collagen (1TCP), urinary hydroxyproline, insulin-like growth factor (IGF-1), carboxy- terminal propeptide of type 1 collagen (P1CP) and others [2, 16]. In this article we will discuss the activity of the two biomarkers DPD and BSAP as they have been more commonly utilized in the literature.

Bone turnover markers have also been used to monitor the effectiveness of bisphosphonate therapy in the treatment of CNA [2, 12–15]. Bisphosphonates have been shown to successfully reduce the levels of these markers [2, 14]. This reduction has led to the investigation of bisphosphonates as a potential medical therapy for CNA. Other medical therapies, such as calcitonin, have been investigated for treatment of CNA; however, none are more extensively studied than bisphosphonates. Indeed, in a recent study of the various pharmacological agents utilized for acute CNA, only eleven original studies were identified with ten of them discussing bisphosphonates and one discussing calcitonin [16]. In a majority of the studies related to use of bisphosphonates in acute Charcot foot, pamidronate has been shown to reduce the markers of Charcot activity like skin temperature, pain, edema, and bone turnover markers, but the quality of evidence is weak [19].

The idea of using pharmacological treatments in acute CNA patients is to transition to post acute or chronic stage quicker which could be beneficial in clinical settings. If such treatments work, as physicians we can make difference in patient's quality of life. Patients can have decreased period of immobilization and offloading, less foot deformity, if deformity is present quicker surgical reconstruction, less loss of limbs and even death. The assumption made here is that the levels of these bone markers correspond to the severity of the patient's disease. There have been studies demonstrating increased bone turnover

marker levels in acute CNA, however to our knowledge no specific study exists that specifically studied BSAP and DPD:CRT levels in acute and chronic CNA phase. The purpose of our study was to compare levels of BSAP and DPD:CRT bone markers in acute and chronic CNA patients to determine if they accurately reflect severity of CNA. We hypothesized that levels of BSAP and DPD:CRT biomarkers would be higher in acute CNA and lower in chronic CNA. If true, it would suggest that by decreasing the levels of these bone turnover markers via pharmacological therapy in acute CNA, we could avoid CNA related major lower extremities complications.

2. Materials And Methods

We performed a retrospective cohort study one of 41 patients from January 2005 to January 2007, who were diagnosed with acute or chronic Charcot neuroarthropathy. Institutional electronic medical record system was utilized to gather data of patient's demographics details, radiographs, physical examination records, pedal temperature in degree Celsius and levels of biomarkers. The data was recorded in password-protected excel spreadsheet provided by institution including age, laterality, site, temperature difference and biomarkers values without patient identifiers. Reviewing baseline radiographs, levels of biomarkers BSAP and DPD:CRT and examining pedal temperature difference in affected and unaffected foot of suspected CNA patients was standard of care in Charcot clinic by the principle author.

The patient population in this study was mainly Caucasian who were referred to Charcot clinic from primary care providers for further evaluation of worsening of the foot deformity or self-referral with chief complaint of edema, warmth or foot deformity. The study was held in an outpatient hospital center specialized in complicated wounds and Charcot deformity located at the St. Luke's University Health Network Hospital in Quakertown, Pennsylvania, USA. Sample size of this study was determined based on the patients who met our inclusion criteria within the identified time frame. Inclusion criteria of acute CNA participants were combination of following findings: patient with unilateral red, hot, swollen intact foot that was not due to recent trauma or cellulitis, evidence of radiographic bone fragmentation, dislocation or subluxation in setting of diabetic peripheral neuropathy, pedal temperature difference of 2 degree Celsius or greater compared with the same site on the contralateral foot. Inclusion criteria of chronic CNA patients were stable intact foot with no evidence of erythema or warmth, radiographic evidence of bone remodeling and disorganized joint architecture in setting of diabetic neuropathy, pedal temperature difference of less than 2 degrees Celsius. Exclusion criteria were mainly CNA patients with active wounds, osteomyelitis or advancing cellulitis, and partial or complete amputation of either limb.

All the patients included in this study were diagnosed with peripheral neuropathy. Peripheral neuropathy was defined as inabilities to sense light touch at any of the seven locations on the foot (dorsal mid foot, plantar surface of the first, third, fifth metatarsal heads; medial and lateral mid foot and central hind foot) using a single-thickness (5.07/10-g) Semmes-Weinstein monofilament [20].

To confirm the presence of local foot inflammation due to CNA, all the patients had pedal skin temperatures assessed with portable infrared surface thermometer, OMEGA Engineering Inc., Stamford,

Connecticut, USA, (Fig. 1.). Patients were asked to remove shoe gear/cast, socks, hose and rest comfortably on an examination chair for at least 15 minutes in temperature-controlled room. Principal authors standard protocol to perform skin surface temperature assessment at the suspected CNA sites either medial or lateral ankle, midfoot, calcaneus, or the first metatarsal phalangeal joint and compare to contralateral limb. An acute CNA was with skin temperature of the affected foot 2 degree Celsius or greater than the contralateral unaffected foot. Chronic or post acute CNA was determined when skin temperature of affected foot was less than 2 degree Celsius compared to contralateral unaffected foot.

Each patient had serum blood and urine chemistries determined at baseline initial visit to Charcot Clinic. Blood samples were extracted from a vein in the arm using a hypodermic needle via phlebotomist at the hospitals outpatient Laboratory. Serum samples of bone formation marker BSAP were evaluated via immunoassay by Quest Diagnostics (Lyndhurst, New Jersey, USA). Urine samples of bone resorptive marker DPD:CRT were evaluated via immunoassay by LabCorp (Burlington, North Carolina, USA). Reference ranges for DPD:CRT used was 2.3–7.4 nmol/mmol and BASP was 7.5–31.6 µg/L according to the laboratories utilized.

Given that this was an exploratory study, we did not determine minimum sample size for statistical power purposes, but instead used all available data during our designated time period. Due to the unbalanced subgroup sample sizes and skewed continuous outcomes, we conducted separate Mann Whitney rank sums tests to compare acute and chronic CNA patient. We used SPSS version 25 (Armonk, NY: IBM Corp) to analyze our data, with $p < .05$ denoting statistical significance for all outcomes, and no adjustment for multiple comparisons.

3. Results

A total of 31 patients (76%) with acute CNA and 10 (24%) with chronic CNA met our inclusion criteria. Tables 1 and 2 present descriptive information for chronic CNA patients regarding pedal temperature difference versus DPD:CRT and versus BSAP, respectively. Both tables also present skin temperature (Celsius) of the affected limb. Tables 3 and 4 present the same descriptive information for acute CNA patients. Figures 2 and 3 present a scatterplot of pedal skin temperature versus DPD:CRT and BSAP, respectively, in chronic CNA patients. Figures 4 and 5 present the same scatterplot of outcomes for acute CNA patients. There was no significant difference between acute and chronic CNA patients in either levels of DPD:CRT ($p = 0.46$) or BSAP ($p = 0.92$). Neither BSAP nor DPD:CRT were associated with disease severity.

4. Discussion

The major finding of our study is that bone turnover markers BSAP and DPD:CRT, levels were not significantly different in acute versus chronic CNA patients, as determined via pedal skin temperature. Therefore, we believe our results suggest that utility of BSAP and DPD:CRT biomarker levels in monitoring CNA severity to initiate pharmacological treatment is questionable. Though there have been few studies

stating bone formation markers were significantly higher in CNA. Gough and co-workers found that alkaline phosphatase was significantly higher in patients with Charcot arthropathy compared to a control group [15]. In their study control groups were patients diagnosed with diabetes with no foot complications and non-diabetic patients. However, they also found there was no difference between levels of alkaline phosphatase in acute vs. chronic Charcot disease.

Petrova et al., have shown that serum C-terminal telopeptide, but not alkaline phosphatase, was significantly higher in people with Charcot osteoarthropathy at presentation than in people with diabetes [21]. The observed lack of increased levels of bone alkaline phosphatase in response to the raised C-terminal telopeptide suggests that, in people with Charcot osteoarthropathy, there may be adynamic bone, related to the presence of diabetic neuropathy, which could impair the regulation of bone remodeling [22, 23]. Alternatively, it is possible that these systemic bone turnover markers fail to represent the extent of the localized bone destruction that takes place in the affected Charcot foot. Petrova et al., in their study has shown higher levels of markers of bone turnover in acute Charcot osteoarthropathy, the lack of longitudinal changes at the time of resolution indicated that bone turnover markers may not be useful in monitoring the activity of the Charcot foot [21]. In the present study we found neither of the bone turnover markers were of substantially different in acute vs. chronic CNA, so the question remains whether we can truly use bone turnover marker levels to monitor pharmacological effectiveness.

Nevertheless, selected authors have utilized DPD:CRT and BSAP as a means of showing the efficacy of bisphosphonate therapy in CNA [2, 14, 17]. In their study of 6 patients, Selby et al., showed significant reduction of alkaline phosphatase as well as significant reduction in temperature difference between affected and unaffected limbs [14]. Jude et al., in their double-blind randomized controlled trial of 39 patients showed significant reductions in DPD:CRT and BSAP after a single infusion of 90 mg pamidronate [2]. Anderson et al., also reported significant reduction in serum alkaline phosphatase after pamidronate infusion [17]. On theoretical grounds, bisphosphonates therapy may have clinical benefits, but the results of published studies are inconclusive. There is little evidence to support the use of bisphosphonates as part of the routine management of patients with diabetes complicated by acute CNA [24]. Use of bisphosphonate is associated with longer time to resolution of CNA. Therefore, offloading and immobilization still remains the mainstay of CNA treatment.

In the present study non-significant difference in bone turnover marker levels in acute vs. chronic CNA could be due to in part to the small overall sample, as well as there only being 10 patients in chronic CNA group. Chronic CNA patients were limited in the given time frame due to not meeting inclusion criteria. As a result, this study may have been underpowered and could have lead to false negative findings. Furthermore, we merely evaluated a systemic biomarker levels that could have had negative impact on the observed results. The biomarkers in question might only be produced locally around the inflamed bones in the foot, which means that the signal on a systemic level can be difficult to register. Pearson et. al., have shown in their study that patients with an acute Charcot foot the concentration of bone markers, OPG, ALP and CTX, was higher in sera from the dorsal vein of affected foot when compared to controls i.e. non-diabetic [25]. Additionally, we did not look at hepatic and renal function of patients in the present

study. Disease specific discrepancies could have reflected differences in the production rates of these bone turnover markers in CNA patients. In future studies, it would be interesting to investigate the levels of BSAP and DPD:CRT in acute and chronic CNA patients with larger sample size in patients with and without specific comorbidities such as renal and liver disease and how it could potentially have effect on the level of bone turnover markers.

5. Conclusion

To our knowledge, Gough et al., is the only group to have studied bone turnover marker levels in acute and chronic phases of CNA [15]. Several studies have evaluated biomarkers levels in acute CNA in which handful of them have shown increased activity of such bone turnover markers. In conclusion, our data suggest that BSAP and DPD:CRT are not directly correlated with disease severity. This is an important finding prior to begin pharmacological treatment in acute CNA to monitor its effectiveness if such markers are used as a reference.

Declarations

Ethics Approval and consent to participate:

The information recorded by the investigator for this study in such a manner that the identity of the human data cannot readily be ascertained directly or through identifiers linked to the subjects. The investigator does not contact the subjects, and the investigators will not re-identify subjects. Therefore St. Luke's University Health Network Hospital IRB committee exempted this study from IRB review.

Consent to publication: Not applicabl

Availability of data and material:

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

Competing interests:

Komal M Patel, Brent Bernstein, Brandy Grahm and Jill Stoltzfus declare that they have no completing interest. None of the authors have competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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Authors' contributions

KP: Submitted the manuscript to IRB for exemption. Participated in data abstraction and analysis. Scripted the final draft of this manuscript.

BG: Formulated the original artwork of the study and helped with data collection, coauthor the final draft.

BB: Conceived of the study and participated in its design and coordination along with assisted to draft the manuscript.

JS: Participated in the design of the study and performed the statistical analysis for this study.

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Tables

Due to technical limitations, Tables 1-4 are provided in the Supplementary Files section.

Figures

Figure 1. OMEGA Surface Temperature Scanner (OMEGA Engineering, Inc., Connecticut)

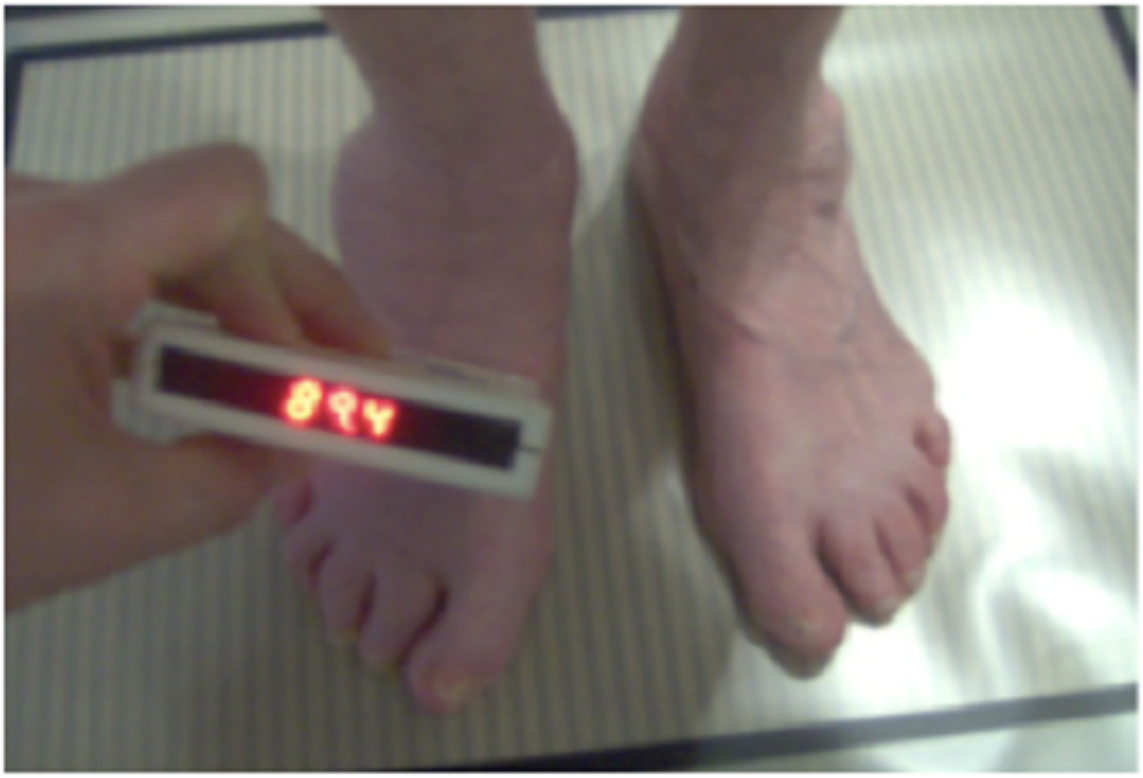

Figure 1

Figure 2. Chronic CNA: Pedal Temparature versus DPD:CRT (nmol/mmol)

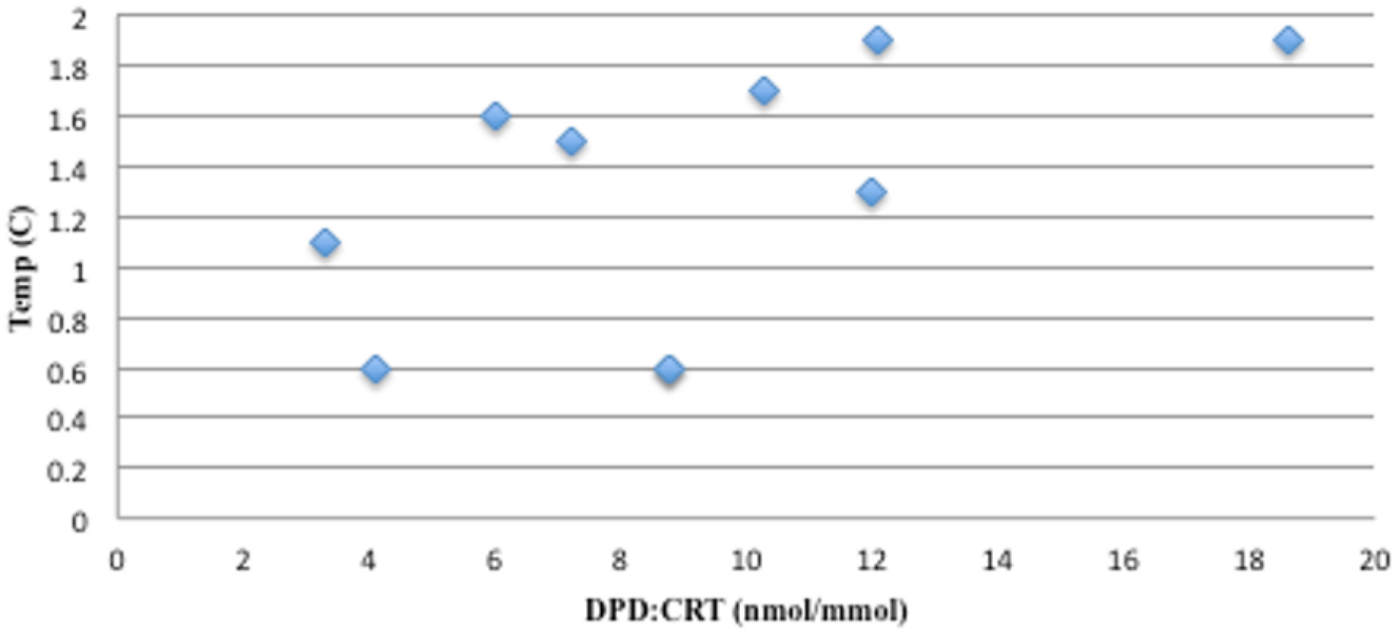

Figure 2

Figure 3. Chronic CNA: Pedal Temparature versus BSAP (µg/L)

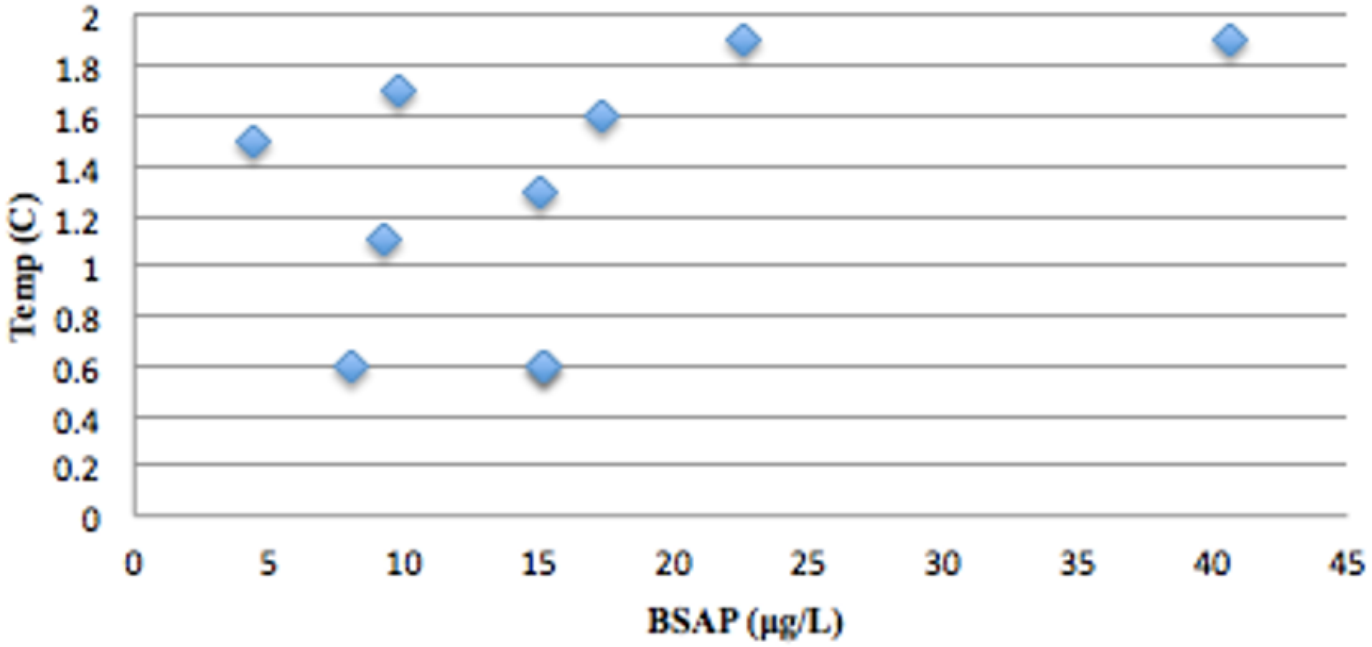

Figure 3

Figure 4. Acute CNA: Pedal Temparature versus DPD:CRT (nmol/mmol)

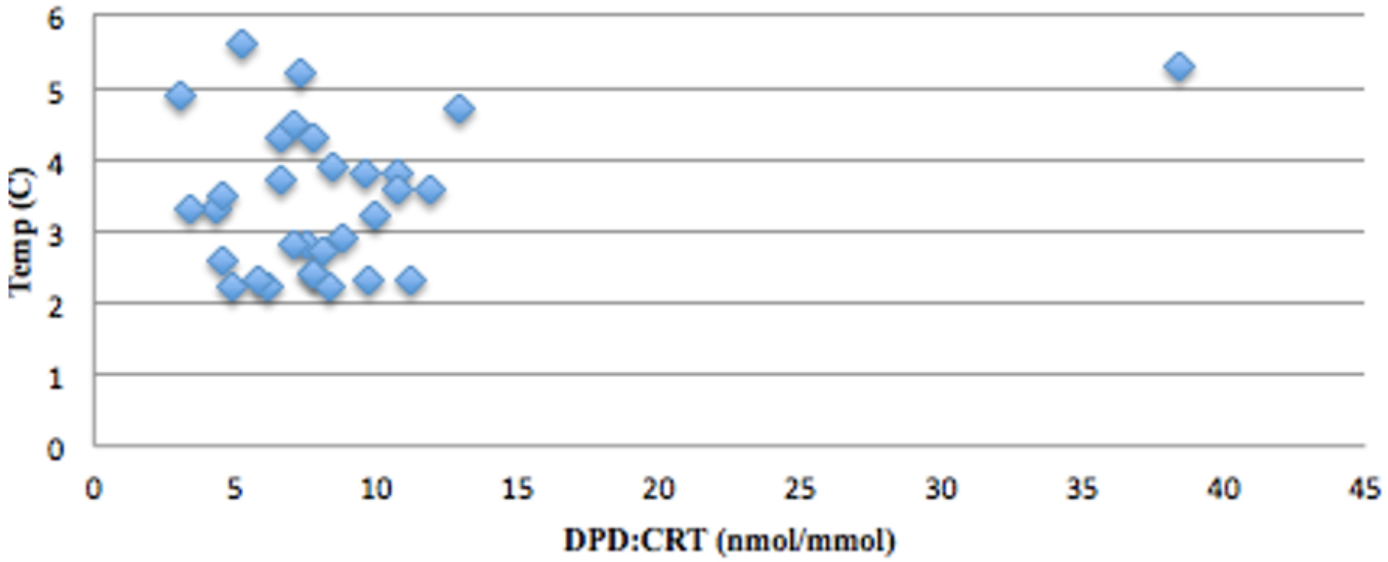

Figure 4

Figure 5. Acute CNA: Pedal Temparature versus BSAP (µg/L)

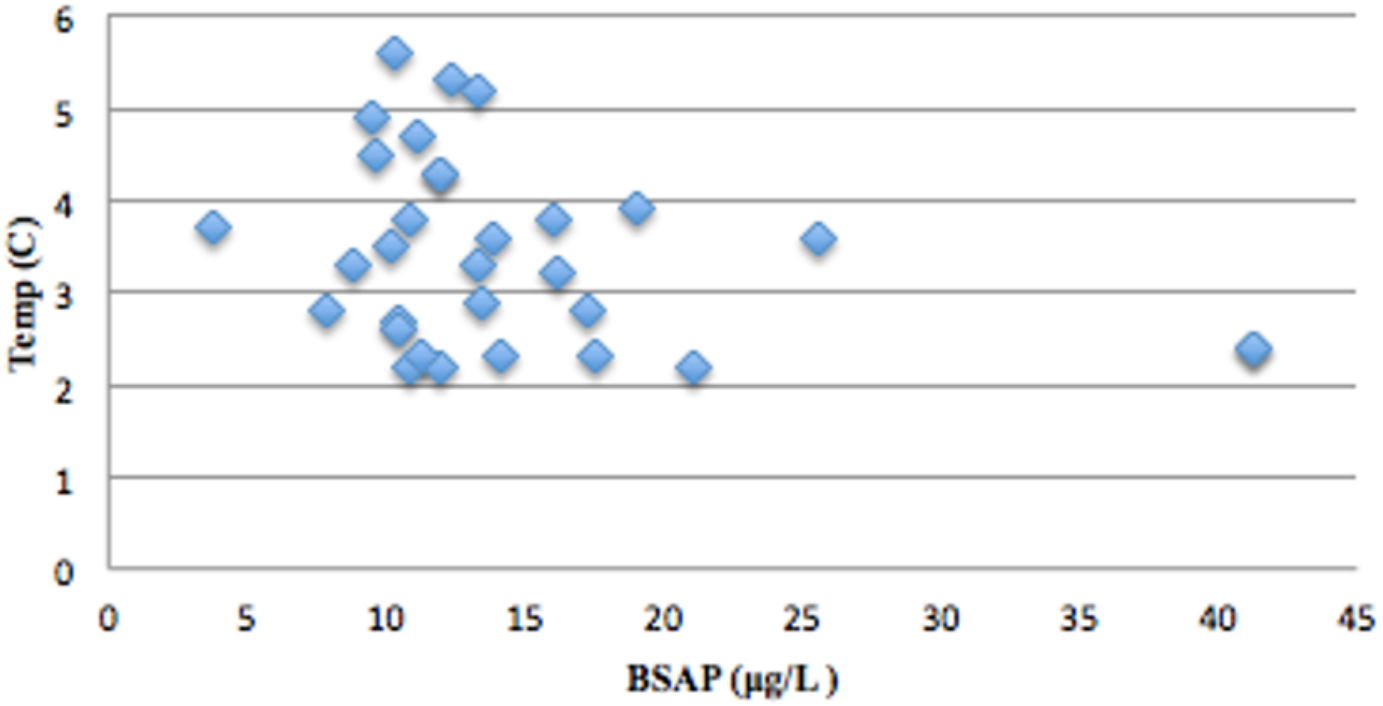

Figure 5

Supplementary Files

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