Differential Abatement of Inflammation And Osteoclastogenesis In Active Charcot Neuroarthropathy: Mechanistic Insights From Randomized Controlled Study

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Abstract

Aims

Inflammatory osteolysis is the sine-qua-non of active Charcot neuroarthropathy (CN). This study is aimed to provide mechanistic insights into pathogenesis of CN by exploring the role of methylprednisolone or zoledronate for remission of active CN.

Methods

Thirty-six patients with active CN (temperature difference >2°C from normal foot) were evaluated for time to remission. Patients were off-loaded with total contact cast and randomised to receive either methylprednisolone (1gm), zoledronate (5mg) or placebo (100ml normal saline) once monthly infusion for three consecutive months. Change in inflammatory cytokines (TNF-α, IL-1β), bone turnover markers (P1NPand CTX) and bone mineral content (BMC) was assessed.

Results

Time to remission of active CN was 19.4 ± 2.8 weeks with methylprednisolone compared to14.6 ± 4.4 and 13.5 ± 2.9 weeks ($p<0.05$) with zoledronate and placebo, respectively. TNF-α levels reduced by 27% ($p=0.03$) with methylprednisolone,18% with zoledronate and 16% with placebo ($p>0.05$), while IL-1β reduced by 30% ($p=0.02$) with methylprednisolone, 21% with zoledronate and 16% with placebo ($p>0.05$). P1NP decreased by 5%, 24% and 19% with methylprednisolone, zoledronate and placebo, respectively. CTX decreased by 35% and 11% with zoledronate and placebo respectively but increased with methylprednisolone (18%) ($p>0.05$).

Conclusion

Effective suppression of either inflammation with methylprednisolone or osteoclastogenesis with zoledronate do not translate into earlier remission of active CN. The role of non-cytokine and non-inflammatory pathways for the activation of osteoclastogenesis in CN is purported.

Introduction:

The etiopathogenesis of Charcot neuroarthropathy (CN) is intriguing since its early description in 1868. Early elucidation for the causation of CN with the neurotraumatic and neurovascular theories were accurate in their times.[1, 2] Of late, conceptual understanding of CN has evolved after description of the role of osteoclastic resorption of foot bones by activation of receptor activator of nuclear factor kappa-B (RANK).[2, 3] The activation of RANK by ligand for RANK (RANKL) occurs as a result of recurrent trauma to an insensate foot inciting a pro-inflammatory cascade of multiple inflammatory cytokines locally, the most common being tumour necrosis factor-α (TNF-α), interleukin-1ß (IL-1β) and interleukin-6 resulting in a local ‘cytokine storm’.[4, 5] In addition, non-inflammatory factors including hyperglycemic milieu by increasing advanced glycosylation end products (AGEs), and autonomic neuropathy by causing a

decrease in calcitonin gene-related peptide (CGRP) and endothelial nitric oxide synthase, can upregulate the RANKL/ NF-κB pathway.[6–8] There is some evidence that suggest a causal role for genetic polymorphisms in RANKL-RANK-OPG genes and autoantibodies against post-translationally modified collagen in the development of CN.[9, 10] Thus, both the inflammatory and non-inflammatory pathways of RANK activation have been proposed for inciting osteoclastogenesis in active CN of foot. At the same time, RANKL-independent osteoclastogenesis mediated through TNF-α and hyperglycemic milieu de novo has also been demonstrated in patients with CN.[3, 11]

The gold standard treatment for the management of active CN is total contact cast (TCC).[12] But TCC has inherent limitations including worsening of bone mineral density (BMD), cast-related tissue injury and prolonged immobilization duration (6 to 12 months).[13] Anti-resorptive agents that target osteoclastogenesis by RANK inhibition are logical therapeutic agents for the cessation of resorption in active CN. Previously, bisphosphonates (pamidronate [14], alendronate [15], zoledronate (ZN) [16], calcitonin [17] and denosumab [18] have been evaluated for their role in active CN. Studies with these agents have evoked mixed results, as few earlier studies demonstrated benefits with calcitonin and pamidronate mostly in terms of parameters like symptom score and bone turnover markers (BTMs); but importantly, time to remission or total immobilization time were not evaluated. Alendronate showed a non-significant effect on temperature difference [15] while Zoledronate showed significantly prolonged time to clinical remission as compared to placebo.[16] Recently, denosumab was found to be effective in reducing the time to fracture healing and clinical resolution of active CN as compared to the historical controls. But repeated doses may be required over the long-term which is undesirable for active CN.

Therefore, considering the recent understanding of etiopathogenesis of CN and significant limitations of prior studies, we planned an interventional, three parallel-arm, proof-of-concept study with an anti-resorptive agent, anti-inflammatory agent and a placebo in addition to TCC as the primary treatment modality in patients with active CN.[19] Methylprednisolone was chosen as its use in rheumatoid arthritis (RA), which is also characterized by cytokine-induced inflammatory osteolysis akin to CN, has been shown to have beneficial effect on clinical remission in RA despite its known adverse effects on bone metabolism.[20] The role of anti-inflammatory agents for curtailment of inflammation in active CN, though proposed, has not been evaluated.[3–5, 21] We preferred to use a monthly pulse instead of a prolonged daily dose because CN is a medical emergency and requires early abatement of inflammation. Zoledronate was included in the study protocol as data with ZL in active CN is still contentious despite it being the most potent bisphosphonate available (100 times more than pamidronate). We aimed to assess the impact of anti-resorptive and anti-inflammatory therapy on the patient-centric outcome of time to remission and the differential effects of these agents on inflammatory cytokines and BTMs to gain novel insights concerning the pathophysiology of active CN.

Patients And Methods:

The study enrolled participants attending the multi-disciplinary foot clinic at a tertiary care centre in India. A total of 143 participants with CN were diagnosed during the study period, and 36 were finally recruited

into the study protocol (Fig. 1). Active CN of foot was defined as a localised swelling, erythema and temperature difference exceeding 2°C compared to a similar site of opposite foot.[14,16] Chronic CN was considered in the presence of peripheral neuropathy with or without fracture/dislocation or prior history suggestive of active CN. Participants fulfilling the criteria for active CN in the setting of chronic CN were considered as “active on chronic” CN. Those with self-reported diabetes or those already on treatment were included. Participants with pedal ulcer (Infectious Disease Society of America grade 2 or more), osteoporosis (T score <-2.5 at lumbar spine or hip), gout, active peptic ulcer disease, steroid intake in the last three months, estimated glomerular filtration rate (eGFR) < 45 ml/min/m^2, active dental caries or invasive dental procedure, peripheral vascular disease (ABI < 0.9), bilateral foot involvement and those who had recently received antiresorptive agents (in the prior 12 months), were excluded.

The diagnosis of active CN was corroborated by X-ray and/or magnetic resonance imaging (MRI) (3T scanner Siemens MagnetromVerio). Sanders-Frykberg classification was used for anatomical grading and localization of the involved site of the foot. The study was approved by the Institutional Ethics committee, PGIMER (IEC/2016/2276) and written, informed consent obtained from all participants. The trial is registered at ClinicalTrials.gov: dated 20/09/17; NCT03289338

Clinical details regarding duration of symptoms, inciting event, diabetes duration and co-existing microvascular and macrovascular diabetic complications were recorded. Peripheral neuropathy evaluation included vibration perception threshold (VPT > 25 mV abnormal) by biothesiometer - Vibrometer, 10-g monofilament (both Diabetik Foot Care, Madras Engineering Service, India) perception at 5 standardized plantar sites and ankle reflex. Foot temperature was measured by infrared dermal thermometry (FLIR Systems Inc, Orlando, USA) with a thermal sensitivity of < 0.15°C and range of detection from – 20°C to 250°C. Bone mineral content (BMC) in gram and BMD (g/cm^2) at the region of interest (ROI) of foot were quantified with Dual Energy X-ray Absorptiometry (DEXA, Hologic 6.0, Model-Discovery A, S/N 87292). The involved foot was scanned from toes to heel. ROI was drawn manually and BMC quantified.[22]

Blood sample for biochemistry, bone turnover markers (BTMs) and inflammatory cytokines were collected after an overnight fast (8–10 hours) at 0800-0900h from the antecubital vein. BTMs, namely procollagen I intact N-terminal (P1NP) and carboxy terminal collagen crosslink (CTX) peptides were estimated by electrochemiluminiscence assay (ECLIA) (Roche COBAS600, Germany) with an inter-assay coefficient of variation (CV) of 1.7 to 3.2%, intra-assay CV of 4.1% for P1NP and inter-assay CV of 1.2 to 4.7%, intra-assay CV of 1.9% for CTX. Inflammatory cytokines (TNF- α, IL-1β) were estimated by human high sensitivity ELISA kits (Thermofischer scientific BMS223HS, BMS224HS) with intra-assay CV 6.0% and inter-assay CV 7.4% for TNF- α and intra-assay CV 6.7% and inter-assay CV 8.1% for IL-1β. Intact parathyroid hormone (iPTH) and 25(OH)D were measured by ECLIA using commercially available kits (Elecsys 2010, Roche diagnostics, Germany), ESR by Westergren’s method, high sensitive C-reactive protein (hsCRP) by latex enhanced quantitative immunoturbidimetric assay (Siemens healthcare diagnostic Inc.) with intra-assay CV 2.9% and inter-assay CV 6.8% and procalcitonin by sandwich ECLIA (Elecsys Brahma PCT, Cobas e601) with intra-assay CV 3.5% and inter-assay CV 4.3%.

All participants were provided with standardised non-walking, non-removable fibre-glass TCC for immobilisation till clinical remission. Subsequently they were randomised by one of the investigators (AR) using computer generated randomisation blocks of 3, to receive methylprednisolone 1gm in 100ml normal saline (NS) (Group A), zoledronate 5mg in 100ml NS (Group B) or 100ml NS (placebo) (Group C) administered in once monthly infusions for three consecutive months. All participants were followed fortnightly and change of cast was offered in view of 'pistoning' effect due to reduction of edema. An average of three temperature recordings at the ROI of foot was obtained during each follow-up after cast removal for 30 minutes.

Clinical remission of active CN was defined as a temperature difference < 2°C between the affected foot and a similar site on the opposite foot on two successive follow-up visits two weeks apart.[12, 23] The primary outcome measure was time to clinical remission with the use of either of these agents administered alongwith standard-of-care TCC. Secondary outcomes were change in levels of inflammatory cytokines, BTMs and BMC from baseline. Adverse events (both related and unrelated) were diligently sought and noted in all patients at each follow-up visit.

Statistical Analysis:

Normality of the data for each variable was assessed by Kolmogorov-Smirnov test. Data are expressed as mean ± SD if normally distributed and as median and inter-quartile range if skewed. Student T-test was used to compare the means of two groups for parametric data and Mann-Whitney U test for non-parametric data. ANOVA was used for comparing means of the three groups for parametric data and Kruskal-Wallis test was used for non-parametric data.. SPSS version 22 was used for data analysis and a p-value < 0.05 was considered significant.

Results:

Forty-two participants were enrolled in the protocol. Six were excluded as detailed in Fig. 1. The demographic details, diabetic complications and foot characteristics of the participants at study enrollment are shown in Table 1.

Table 1
Baseline clinical and biochemical parameters of the cohort

Parameter	Group A n = 11	Group B n = 12	Group C n = 13	p value
Age (years)	51.1 ± 4.7	60.9 ± 8.2	59.1 ± 12.4	0.05
BMI (kg/m2)	26.1 ± 4.1	25.9 ± 5.2	24.9 ± 4.2	0.62
Males: Females	6: 5	8: 4	10: 3	0.38
Duration of diabetes mellitus (years)	10.9 ± 6.2	13.2 ± 2.6	12.1 ± 6.4	0.37
Duration of symptoms (months)	3.5 ± 2.1	2.1 ± 1.4	2.7 ± 1.8	0.19
Insensate to monofilament (%)	80	86	100	0.50
Precipitating event (%)	30	38.5	54	0.49
Active disease (%)	65	80	72	0.62
Active on chronic disease (%)	35	20	28	
VPT (mv)	32	29	30	0.46
Right: left foot (%)	60:40	57:43	67:33	0.93
Midfoot involvement (%) (SF III)	73	50	69	0.81
Retinopathy (%)	64	75	70	0.82
Nephropathy (%)	55	67	70	0.59
HbA1c (mmol/mol)	77	79.2	67.2	0.12
HbA1c (%)	9.2 ± 1.9	9.4 ± 1.4	8.3 ± 1.2	0.12
eGFR (ml/min/m2)	73.3 ± 10.2	68.3 ± 17.7	68.2 ± 13.9	0.78
Calcium (mg/dl)	9.42 ± 1.03	8.99 ± 0.71	9.03 ± 0.57	0.66
25(OH)D (ng/ml)	20.08 ± 1.06	25.36 ± 0.98	19.38 ± 1.43	0.90
iPTH (pg/ml)	40.06 ± 2.98	35.21 ± 5.13	48.05 ± 2.33	0.76
ESR (mm/hr)	12 (11-15.75)	13 (12–21)	18 (14–21)	0.10

Group A- Methylprednisolone; Group B- Zoledronate; Group C- Placebo; SF- Sanders Frykberg classification of involvement of region/joint in Charcot foot. Data are expressed in Mean ± SD, percentage expressed in terms of the whole group or median (q25-q75) depending on normality.

Parameter	Group A n = 11	Group B n = 12	Group C n = 13	p value
hsCRP (mg/L)	3.7 (1.87–12.05)	3.6 (2.5–28)	4.0 (2.87–14.60)	0.58
Procalcitonin (ng/ml)	0.04 (0.02–0.10)	0.04 (0.02–0.10)	0.02 (0.02–0.05)	0.47
P1NP (µg/L)	48.7 ± 20.4	76.9 ± 57.5	74.7 ± 39.8	0.31
CTX (ng/ml)	0.39 ± 0.26	0.29 ± 0.12	0.41 ± 0.19	0.15
TNF-α (pg/ml)	0.312 (0.269–0.661)	0.298 (0.255–0.768)	0.473 (0.252–2.230)	0.96
IL-1β (pg/ml)	0.062 (0.048–0.072)	0.058 (0.042–0.088)	0.056 (0.041–0.057)	0.48

Group A- Methylprednisolone; Group B- Zoledronate; Group C- Placebo; SF- Sanders Frykberg classification of involvement of region/joint in Charcot foot. Data are expressed in Mean ± SD, percentage expressed in terms of the whole group or median (q25-q75) depending on normality.

Baseline clinical and biochemical parameters were comparable between the groups (Table 1). The mean time for clinical remission in the whole cohort was 15.5 ± 4.2 weeks. Time to clinical remission was significantly higher in the methylprednisolone group (19.4 ± 2.8 weeks), as compared to either zoledronate (14.6 ± 4.4 weeks, p = 0.01) or placebo (13.5 ± 2.9, p = 0.01). However, there was no difference in time to clinical remission between zoledronate and placebo group (p = 0.98). The trend of fall in temperature between involved region and corresponding similar region of contralateral foot on follow-up are depicted in Fig. 2.

There was a decrease in pro-inflammatory cytokines in all groups. TNF-α levels reduced by 24% (p = 0.05) and 27% (p = 0.03) at 3 and 6 months from baseline with methylprednisolone; 11% (p = 0.22) and 18% (p = 0.16) with zoledronate; 6% (p = 0.19) and 16% (p = 0.08) with placebo. Similarly, IL-1β reduced by 25% (p = 0.04) and 30% (p = 0.02) at 3 and 6 months from baseline with methylprednisolone; 15% (p = 0.20) and 21% (p = 0.19) with zoledronate; 9% (0.17) and 16% (p = 0.08) with placebo, as shown in Fig. 3. There was a non-significant decrease in P1NP in all the three groups, but non-significant increase in CTX with methylprednisolone and non-significant decrease with placebo and zoledronate at 3 and 6 months compared to baseline as shown in Fig. 4. There was a 13% (p = 0.03) and 9% (0.09) reduction of BMC (ROI) with methylprednisolone and placebo, respectively, while 35.8% (p = 0.02) increase in zoledronate BMC (ROI) was observed (Supplementary Table 1).

Among adverse events, flu-like reaction (n = 5, 41.6%) and acute kidney injury (defined as increase in serum creatinine > 0.5mg/dl above baseline or estimated GFR under 30ml/min/m^2) (n = 2, 16.6%) were the common adverse events noted with the use of zoledronate [30]. Worsening of glycemic profile was observed with methylprednisolone. In addition, cast related tissue injury were observed in 2 patients each in all the three groups.

Discussion:

We found that neither MP nor ZL could lead to an early clinical remission as compared to TCC alone in active CN. There was a significant decrease of pro-inflammatory cytokines with MP, but ongoing osteolysis could not be abated, indicating the role of cytokine-independent pathways in the progression of bone destruction, as elaborated in Fig. 5. Similarly, there was significant suppression of osteoclast activity with ZN, but could not translate into earlier remission.

The criteria used to define remission of active CN are based on reduction of signs of inflammation including redness, swelling and normalisation of temperature difference (< 2°C) between both feet, which is the most consistent and objective parameter. These clinical criteria usually correspond to resolution of marrow edema on T2W MRI images and normalisation of radiotracer uptake on TcMDP bone scan at ROI.[24, 25] Therefore, most studies available in the literature have utilised and recommended only clinical criteria to predict the remission. This apparently seems in consonance with the pathogenesis of disease as clinical signs of inflammation are predominantly contributed by preceding local 'cytokine storm' and consequent release of prostaglandins coupled with exaggerated vasoreactivity in response to cumulative minor trauma to an insensate foot. Further, ongoing osteolysis as a result of cytokine-mediated activation of osteoclastogenesis, also contributes to ongoing signs of inflammation. Thus, the diminution of clinical signs of inflammation and stabilisation/cessation of ongoing osteolysis may not always be concordant and usually takes a prolonged period of time varying from 6 to 12 months.

Off-loading the inflamed foot in active CN with a non-walking TCC is considered as the "gold standard" of treatment.[12] A non-walking TCC not only helps by preventing further trauma to the insensate foot thus abating inflammation and subsequent osteoclast activation but is also instrumental in reducing and favourably redistributing the abnormal plantar pressure distribution.[26] However, it usually takes more than 6–12 months for clinical remission.[13] During this long period of non-ambulation, BMD of foot bones may be adversely affected. Therefore, efforts were made to identify pharmacotherapeutic agents that could target the pathophysiology of CN of foot in patients with diabetes.

The recent understanding of pathophysiology of active CN focuses on the role of inflammatory cytokines that are postulated to incite RANKL-NF-κB activation and consequently, local osteoclastogenesis in the affected foot bones. [4, 5, 21] There is some pre-clinical evidence demonstrating reduction in resorption with anti-TNF-α antibodies [27] but no clinical evidence for the use of anti-inflammatory agents in active CN, till date. The current study used methylprednisolone in high dose pulse therapy to abruptly curtail the local 'cytokine storm'. We observed a delay in resolution of active CN with MP, despite cogent suppression of cytokines. Although circulating cytokine levels were assessed, they were regarded as indicative of local cytokine concentration, owing to the fact they were assessed in the same individuals at baseline and end of follow-up. The delay in resolution of clinical activity of CN despite suppression of cytokines can be explained by uninhibited activation of cytokine-independent RANKL pathway, suppression of favourable anti-inflammatory cytokines involved in bone healing (IL-4, IL-10) and worsening of hyperglycemia which, in turn, directly exacerbates the RANKL-NF-κB activity and subsequent osteoclastogenesis.[28] Also, the

increase in bone resorption and decrease in bone formation, resulting in ongoing osteolysis of foot bones could have contributed to a delay in clinical remission.

Lack of substantial evidence regarding efficacy of ZL in achieving clinical remission in active CN may be attributed to the doses used in previous studies (4mg versus 5mg), frequency of admission (single use versus multiple monthly doses), longer lag period and a very modest effect on 'cytokine storm'.[13, 16] Initial studies with alendronate and pamidronate showed gain in terms of improvement in symptom score and suppression of bone turnover but their effect on clinical remission was either not found to be significant or not investigated. A systematic review[29] and few recent studies [13, 16] suggested that bisphosphonates may not reduce time to remission in patients with active CN or even increase the time in cast. The current study shows a remarkable decrease in bone resorption markers and consequent increase in BMC with ZL suggesting effective suppression of ongoing osteoclastogenesis. This could be pertinent in the prevention of deformities and fractures over long-term, due to long retention of the drug in bone matrix But, monotherapy does not seem to be sufficient for clinical resolution as 'cytokine storm' may continue unabated due to only a modest immunomodulatory efficacy of ZL. Similarly, denosumab, a monoclonal antibody against RANK-L, decreased time of fracture healing and clinical resolution of active CN in an open-label trial using historical controls.[18] Although denosumab is a potent anti-resorptive, the drug necessitates long-term usage as there is a high rate of rebound fractures on drug withdrawal. In fact, a recent meta-analysis of randomized controlled trials of all the studied medical therapies for active CN found no difference in outcomes in comparison to struct offloading with TCC.[30]

Periodic analysis of bone turnover markers (P1NP and CTX) suggested maximum osteolysis at baseline in all groups, in accordance with clinical activity of active CN as demonstrated previously.[21] Both P1NP and CTX declined in the zoledronate and placebo groups on follow-up without any significant difference between the groups, suggesting an overall decrease in bone turnover because of off-loading. On follow-up, maximum bone mass accrual at ROI was noted with zoledronate despite a similar alteration in cytokines as compared to placebo, suggesting its direct beneficial effect on bone parameters. A similar improvement in foot BMD has been demonstrated earlier with oral bisphosphonate using DEXA.[15] However, whether the initial gain in BMD persists after the quiescence of clinical activity needs to be assessed in long term follow-up studies. Though teriparatide has been shown to increase foot bone BMD in patients of chronic CN of foot,[22] its efficacy needs to be evaluated in patients of active CN.

Adverse events were noted in all three groups. Patients in the zoledronate group developed a transient flu-like reaction, which recovered with the use of analgesics and supportive care. The incidence of flu-like reaction in the current study was higher than previously reported (31.6%) where any one component of acute phase response (pyrexia, myalgia, headache, arthralgia, influenza-like symptoms) was considered. Two patients (n = 2, 16%) developed acute kidney injury after the second dose of zoledronate which is comparable to the incidence following standard dosing regimen of bisphosphonates (8 to 15%).[31] Cast-related tissue injury was observed in two patients from either group with literature evidence showing an overall incidence of 5.7%. A significant proportion of the patients in the methylprednisolone group

developed worsening of glycemic profile that was managed by intensification of subcutaneous insulin therapy.

The strengths of the current study include proof-of-concept for the use of anti-inflammatory agent in active CN, an RCT design, homogenised use of standard-of-care TCC in all and precisely defined criteria for remission of active CN. The observations from the present study enable proposition of newer aspects of etiopathogenesis of active CN. We perceived certain limitations of the current study including a small cohort and a shorter duration of follow-up. Longer follow-up could provide more patient centric information like fractures, deformities and recurrences, keeping in mind the anticipated benefit of increased BMC due to tendency for long retention of bisphosphonates in the skeleton. RANKL and anti-inflammatory cytokines (IL-4, IL-10), a dorsal venous arch sample (rather than systemic sample) and in-vitro assessment using a bone biopsy sample could provide newer perspectives in the pathogenesis of active CN.

Conclusions

The current study demonstrates that neither amelioration of pro-inflammatory milieu with anti-inflammatory agents like methylprednisolone nor effective suppression of osteoclastogenesis with zoledronate translate into earlier remission compared to off-loading with total contact cast without pharmacological intervention. Newer insights into pathophysiology may pave the way for novel therapeutic targets into the still enigmatic Charcot neuroarthropathy.

Declarations

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No external/internal funding was availed of, for this study.

Author contribution

statement: LD was involved in clinical care and follow-up of the participants and wrote the initial draft of the manuscript. AR conceived the study, designed the study protocol, was involved in clinical care of the participants, editing and revision of the final manuscript. AR also randomized the participants and administered the study drug. PD and AB critically reviewed the study design and edited the manuscript. MP provided relevant radiological expertise. All authors approved the final version of the manuscript.

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Statement: All procedures included in the study were performed after prior ethical approval, consent of the participants and according to local guidelines and regulations

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Figures

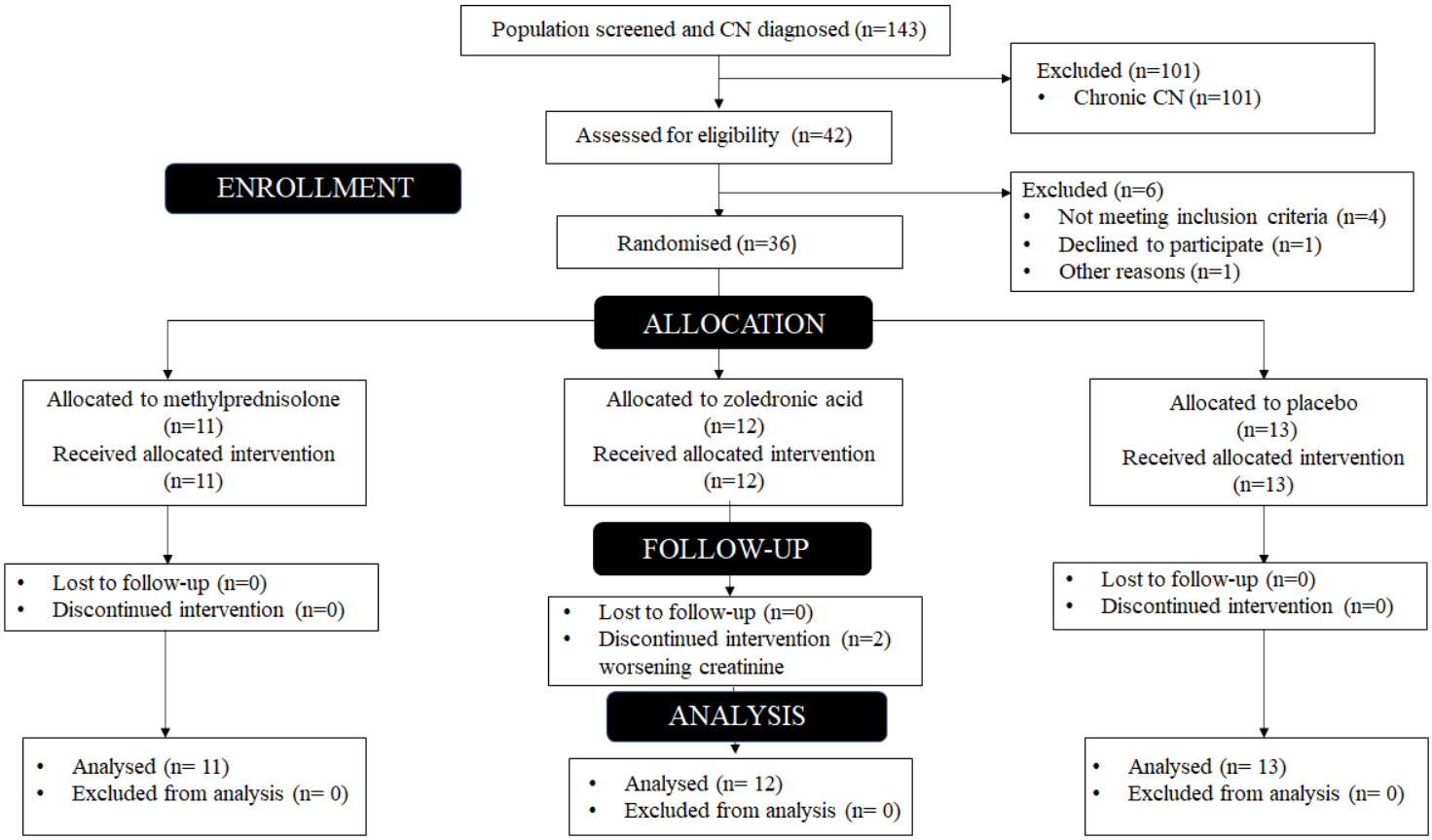

Figure 1

Randomisation protocol as per the CONSORT guidelines (CN- Charcot's neuroarthropathy)

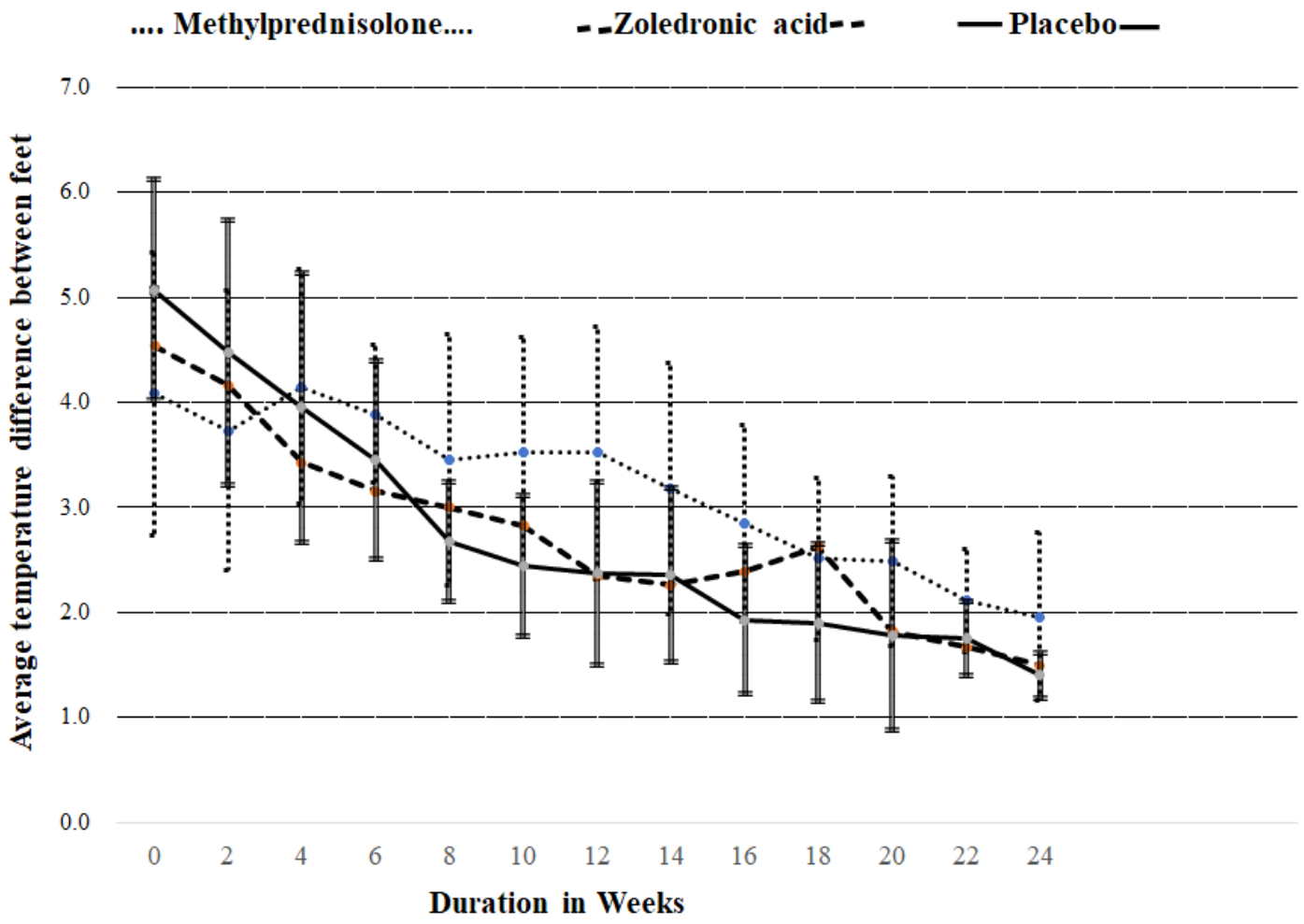

Figure 2

Trends of decline in temperature between corresponding points on the involved and normal foot in the 3 intervention arms

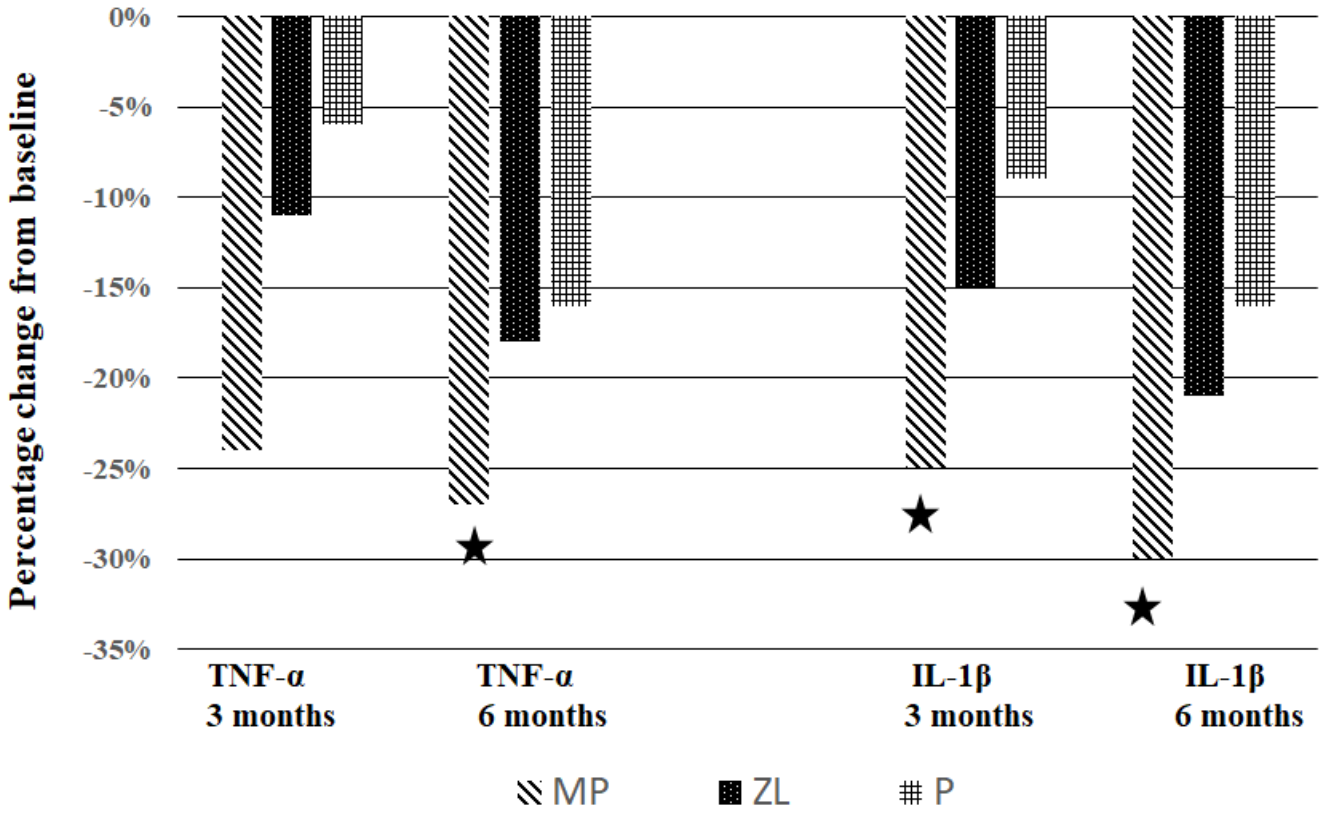

Figure 3

Prospective analysis of inflammatory cytokines in study groups showing changes in TNF-α and IL-1 β at 3 and 6 months (Significant changes from baseline are marked by asterisk; MP- Methylprednisolone, ZL- Zoledronate, P- Placebo)

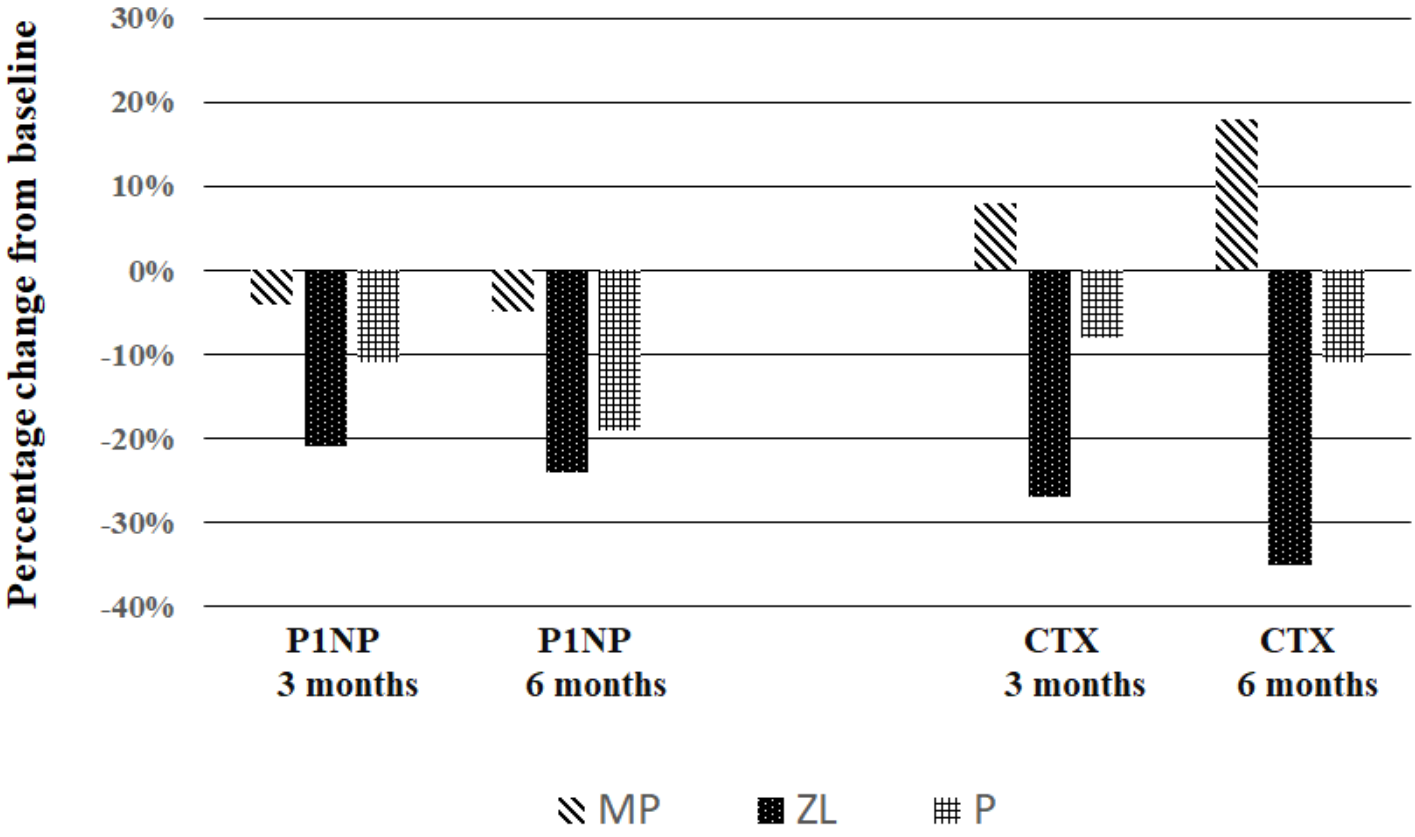

Figure 4

Prospective analysis of bone turnover markers in study groups showing changes in P1NP and CTX 3 and 6 months (MP- Methylprednisolone, ZL- Zoledronate, P- Placebo)

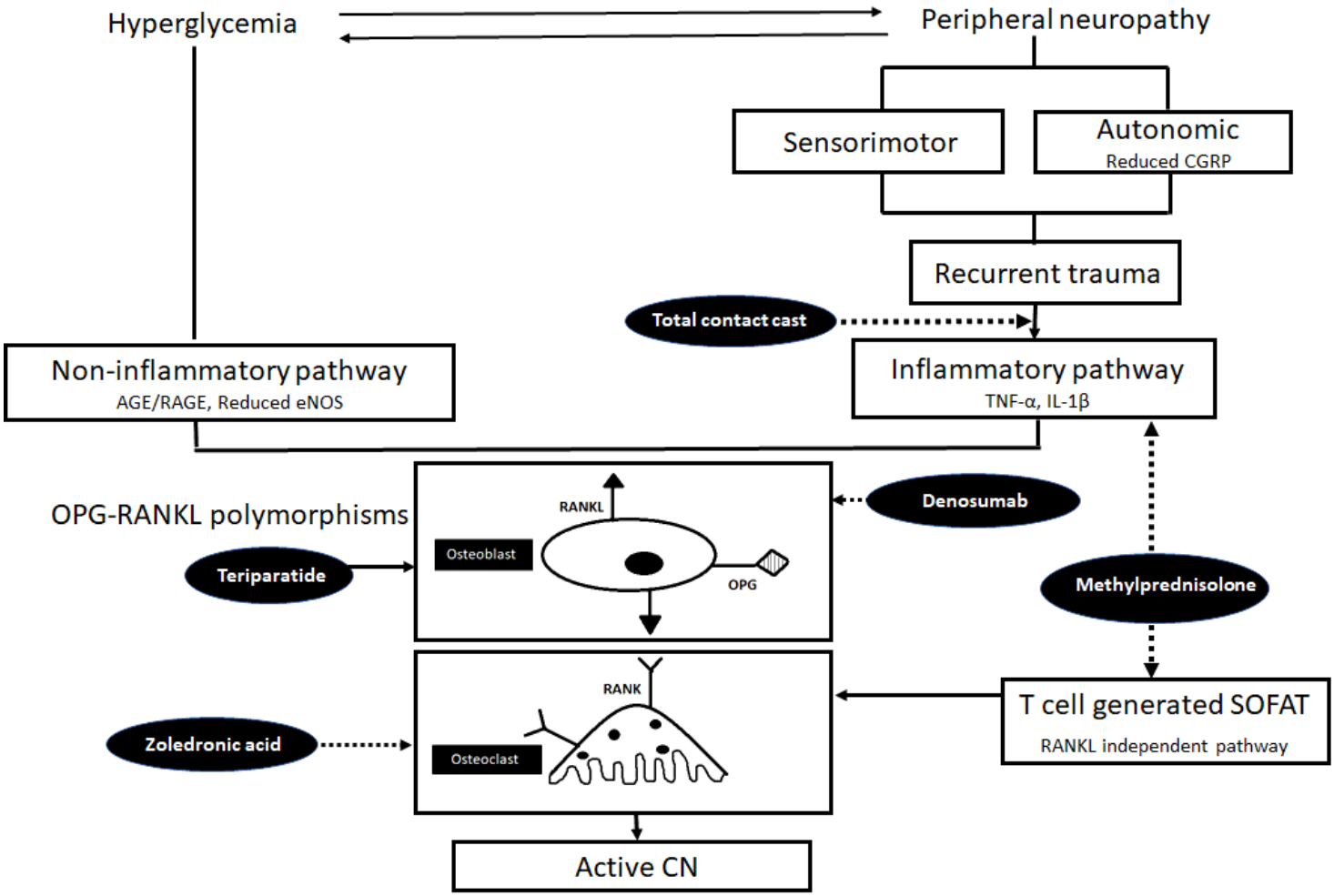

Figure 5

Integrated pathophysiology and interactions between various factors in persons with diabetes implicated for the causation of active Charcot neuroarthropathy and efficacy of various evaluated therapeutic agents AGE- Advanced glycation end products; RAGE- Receptor for advanced glycation end products; eNOS- Endothelial nitric oxide synthase; SOFAT- secreted osteoclastogenic factor of activated T cells; RANKL- Receptor activator of nuclear factor-κβ, OPG- Osteoprotegerin; CGRP- Calcitonin gene related peptide; Charcot's neuroarthropathy (CN) Dashed arrows indicate inhibition and solid arrow indicates activation of the particular target o Total contact cast- Standard of care for remission of active CN o Methylprednisolone- No proven benefit in causing remission of active CN o Bisphosphonates- Equivocal evidence in causing remission of active CN o Denosumab- Proven efficacy for early remission of active CN o Teriparatide- No available evidence in active CN but benefit shown in chronic CN

Supplementary Files

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- SupplementaryTable.docx