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# Role of neuropathy on fracture healing in Charcot neuro-osteoarthropathy

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## Abstract

Charcot neuro-osteoarthropathy (CNO) is a devastating condition affecting most commonly the foot/ankle joint in diabetic patients and may lead to severe deformities and amputation. Peripheral sensory neuropathy seems to be a pre-requisite to the development of CNO. The aim of this review article is to summarise the skeletal effects of the nervous system on bone remodelling and fracture healing of normal and damaged joints and to describe how neuropathy, in the context of modern concept of neuro-osteopathology, is crucial in the predisposition of the patient to develop acute CNO.

**Keywords:** Charcot Neuro-osteoarthropathy, Diabetes, Nerve Fibres, Osteoclasts, Bone Resorption

## Introduction

One of the most insidious complications of diabetes is peripheral neuropathy, which can lead in the lower limb to foot ulcers, Charcot neuro-osteoarthropathy (CNO) and amputation. CNO clinically presents as a red, warm, swollen foot or ankle in a patient with diabetic neuropathy (i.e. neuronal loss). It is characterised by progressive osteolysis, which can lead to multiple fractures, joint destruction and joint dislocation which can result in severe deformity (Figure 1)<sup>1</sup>. This can lead to ulceration, and eventually amputation. The outlook for patients who develop CNO is very poor. Sohn et al reported that over a five year follow-up, 28.3% of diabetic patients affected by CNO died compared with 18.8% with diabetes alone<sup>2</sup>. Thus it is a severely disabling complication of diabetes and its aetiology remains poorly understood.

In 1868, Jean-Martin Charcot gave a detailed description of the arthropathy occurring in tabes dorsalis and this condition bears his name<sup>3</sup>. The association with diabetes was put forward by Jordan when he described a case of CNO in a diabetic pa-

tient<sup>4</sup>. In developed countries, CNO is most commonly encountered in diabetic individuals and affect mostly the foot/ankle joint. However, similar joint deformities occur in other conditions including tabes dorsalis, syringomyelia, leprosy, heavy metal poisoning, congenital sensory neuropathy, alcoholic neuropathy and major trauma to peripheral nerves. Interestingly, in these other conditions, CNO is not restricted to the foot, but can affect the shoulder, elbow, spine, hip and knee joints. The common and required feature between CNO and the other pathologies leading to joint deformities is the presence of nerve damage, which is limited to either the spinal cord or to peripheral nerves.

The aetiopathogenesis of diabetic CNO is still unclear. Two theories have been put forward for the development of this joint condition. The first theory, known as the French theory, was initially proposed by Charcot himself. He suggested that arthritic changes in tabes dorsalis were the results of damage to the central nervous system within the centres that control bone and joint nutrition. He noted that the onset of the arthritic changes was characterised by a massive swelling suggestive of uncontrolled inflammation. Furthermore, Charcot described the onset of CNO before the development of the ataxic gait related to tabes dorsalis. Therefore neurological damage preceded the development of, and was in some way directly responsible for, the changes occurring in CNO. The second theory, known as the German theory, was supported by Volkman and Virchow. They proposed that the changes in bone and joint were a result of a multiplicity of minor trauma, which went unperceived because of the insensitivity of the affected joint. This theory was tested by Eloesser who sec-

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**Figure 1.** Example of a severely deformed Charcot foot. (A) Ankle X-ray and (B) ankle deformity.

tioned posterior nerve root in animals: following a period of activity he found bony changes in 71% of these animals<sup>5</sup>. In more recent years, Finsterbush and Friedman repeated Eloesser's experiments, but casted the animals after sectioning the posterior roots<sup>6</sup>. The authors noted a difference in the response to immobilisation between normal and denervated groups and concluded that trauma, though important, is not a fundamental factor leading to the deterioration of desensitised joints. This is also confirmed by the clinical observation that around two thirds of patients do not remember having injured their foot before developing an acute episode of CNO<sup>7</sup>. Several animal models of neuropathic arthropathy have been developed in cats<sup>5,8,9</sup>, rabbits<sup>6</sup>, dogs<sup>10</sup> and rats<sup>11</sup> in order to elucidate further the cause of this complication. In dogs, animals that underwent both dorsal-root ganglionectomy and cruciate ligament transection developed accelerated degenerative joint lesions as opposed to dogs who had ganglionectomy only<sup>10</sup>. However, it is unclear whether differences observed between the different models reflect species-specific responses to the same basic experimental protocol, whether neuropathic arthropathy develops because of sub-clinical micro-trauma during day-to-day activities, or whether gross trauma is necessary to initiate the process. Nevertheless, overall it may be concluded from these studies that a pre-existing nerve disorder may predispose some individuals with unstable or damaged joints to the rapid development of degenerative joint lesions.

The aim of this review article is to summarise the skeletal effects of the nervous system on bone remodelling and fracture healing of normal and damaged joints and to describe how neuropathy, in the context of modern concept of neuro-osteoarthropathy, is crucial in the predisposition of the patient to develop acute CNO. This review will discuss the innervation of the joint, biology of fracture healing, influence of the nervous system on fracture healing and relevance of neurogenic control on bone remodelling and fracture healing in CNO.

## Innervation of the joint

A joint is a complex anatomical piece comprising different constituents such as synovium, ligaments, tendons, cartilage and bone tissue. All articular structures except cartilage are innervated<sup>12</sup>. Joint nerves are composed of both myelinated and unmyelinated fibres. About 20% of the axons are myelinated. They contained large diameter mechanoreceptors, which are thickly myelinated and are rapid conductors, and smaller diameter, thinly myelinated nociceptors and mechanoreceptors. About 80% of the axons are unmyelinated, half are sympathetic fibres and the rest are sensory fibres<sup>13,14</sup>. Joint tissues contain a variety of nerve endings and the most abundant is the free nerve endings<sup>15</sup>. Synovium, ligaments and tendons are richly innervated by sensory neurons that are mechanically sensitive and activated by tensile stresses<sup>16,17</sup>. Thus, joint rotations cause capsule loading that activates sensory neurons in the joint capsule and ligaments. The resulting activity signals the nervous system that the joint is near its limit of rotation. The existence of a reflex arc from ligaments to the relevant muscles in humans has been demonstrated and participates in maintaining joint stability<sup>17</sup>.

Immunohistochemical analyses have shown that the skeleton is more widely innervated than previously thought and several neuropeptides and other nerve signalling molecules have been shown to be expressed by skeletal nerve fibres<sup>18</sup>. Sensory nerve fibres are present in the periosteum, cortical bone and bone marrow and are particularly rich at the osteochondral junction of the growth plate<sup>19-21</sup>. Furthermore, during skeletal development, skeletal nerve fibres are particularly abundant in areas of bone formation, and in agreement with this observation, sprouting of nerve fibres has been observed during fracture healing<sup>22,23</sup>.

About one third of the unmyelinated free nerve endings in

joints contain substance P, calcitonin gene-related peptide (CGRP), or vasoactive intestinal peptide (VIP)<sup>24</sup>; peptides that are potent inflammatory modulators. Substance P is a vasodilator, which increases capillary permeability, induces mast cell degranulation and is a potent leukocyte chemotactic agent. It is also mitogenic and chemotactic for endothelial cells and fibroblasts<sup>25,26</sup>. CGRP is a potent vasodilator, a mitogen for endothelial cells and a potent inhibitor of insulin-mediated glycogen synthesis<sup>27</sup>. VIP is found mainly in parasympathetic nerves in peripheral sensory neurons and post-ganglionic sympathetic nerve fibres in bone<sup>28,29</sup>. VIP is mitogenic for keratinocytes and downregulates lymphocyte proliferation<sup>29</sup>. VIP has been shown to be very effective in the treatment of collagen-induced arthritis in mice, where it may act by shifting the balance of Th1 and Th2 cells in favour to Th2<sup>29</sup>. Acting together, these nerve-derived peptides can significantly modulate the inflammatory response and their effects have been extensively studied in neurogenic inflammatory conditions such as rheumatoid arthritis. Besides this neurogenic inflammatory effect, there is a growing body of evidence that the nervous system is directly involved in bone remodelling. In the last twenty years, several studies have demonstrated that both osteoblasts, the bone-forming cells, and osteoclasts, the bone-resorbing cells, express functional receptors for these neuropeptides<sup>18</sup>. In bone, the main role of CGRP and VIP is to inhibit bone resorption and stimulate bone formation. *In vitro*, substance P has been demonstrated to both stimulate and inhibit bone formation and increase bone resorption.

## Biology of fracture healing

Healing of a fracture is a complex phenomenon divided into three distinct phases: (i) an inflammatory phase, (ii) a proliferative phase and (iii) a remodelling phase<sup>30</sup>. The inflammatory phase starts initially with bleeding from the damaged bone ends and from the associated soft tissues and a clot forms soon between the fragments. The soft parts in the region show evident signs of acute inflammation with vasodilatation and the exudation of plasma and leucocytes. Polymorphs, mast cells, lymphocytes, monocytes and macrophages make their appearance soon and the process of clearing up of the debris begins. Monocytes proliferate, differentiate into macrophages, indispensable for removal of the necrotic bone debris and clot and start cleaning the zone between the two bone ends. At the same time, necrotic bone cells and local factors (cytokines and growth factors) produced by inflammatory cells stimulate the migration of mesenchymal stem cells from the cambial layer of the periosteum, the endosteum and the bone marrow<sup>31</sup>. Mesenchymal stem cells respond to the inflammation by entering the osteogenic or chondrogenic lineage. The proliferative phase starts as soon as 24 hours after the trauma and can last at least for three weeks. Endochondral bone formation always takes place closest to the fracture site where the oxygen tension is low and vascularity is disrupted. Intramembraneous bone formation, on the other hand, always occurs distal to the disjunction where intact vasculature remains present and is often

associated with the formation of an unorganised collagenic bone matrix also known as woven bone. The mechanical stability of the fractured bone markedly affects the fate of the progenitor cells, with stabilised fractures healing occurs with virtually no evidence of cartilage, whereas non-stabilised fractures produce abundant cartilage at the fracture site<sup>32</sup>. Once mesenchymal stem cells have committed to the chondrogenic or osteogenic lineage, chondrocyte and osteoblast differentiation takes place. Directly overlying the site of the fracture, the ends of the original bone have decreased perfusion caused by disrupted vascularity and necrosis occurs. In this central hypoxic region, mesenchymal stem cells differentiate into chondrocyte and endochondral ossification is initiated. The tissue that forms as the cell population expands is referred to as the callus, and differentiation of mesenchymal stem cells into chondrocytes occurs within the callus with the process initiating in the most central avascular region. The calcified cartilage, which acts as a template for bone formation, is synthesised by the most terminally differentiated hypertrophic chondrocytes that are contributing to the mineralisation of the tissue. Intramembraneous ossification occurs distally to the fracture site, flanking chondrocytes undergoing endochondral ossification. This process, which proceeds in the zone of injury where blood supply had been better preserved, is characterised by the differentiation of mesenchymal stem cells into osteoblasts that directly lay down a new mineral without a cartilage intermediate<sup>33</sup>. The molecular signalling pathways involved in the initiation and tissue morphogenesis that occurs during fracture repair are only superficially understood. Nevertheless, the most notable regulators of this process are the bone morphogenic proteins that belong to the transforming growth factor-beta superfamily, Hedgehog and Wnt proteins, fibroblast growth factors, and insulin-like growth factors<sup>33</sup>. The remodelling phase marked the end of the healing process. This phase is characterised by the replacement of the initial woven bone matrix by organised lamellar bone. It is the critical final step in achieving an anatomically correct skeletal element and is governed by osteoclasts which are present even at earlier stage of healing, but become dominant in this last phase.

## Influence of the nervous system on fracture healing

Clinical and experimental observations indicate that bone growth, repair and remodelling may be under the influence of the nervous system<sup>34-36</sup>. For example, in amphibians, an intact nerve supply is essential for development and regrowth of amputated limbs<sup>37,38</sup>. The pioneering work of Aro and co-workers provided evidence that an intact innervation is needed for union of the fracture in the rat as the removal of periosteal mechanoreceptors caused a non-union in fibula while sciatic nerve section proximal to the injury led to an incomplete maturation of new bone<sup>34,39</sup>. In humans, neural injuries are known to affect fracture healing. Brain damage appears to stimulate callus formation and fracture healing<sup>40</sup> while spinal injuries and paralysis may cause pathologic fractures and excessive

callus formation<sup>41</sup>. As mentioned earlier, bone and periosteum received both sensory and autonomic innervation containing substance P, CGRP and VIP of which CGRP-containing sensory nerves appears to be the most abundant. CGRP is one of the most potent vasodilator agents identified to date. It also stimulates endothelial cell proliferation, an action particularly relevant in fracture healing. Hukkanen and co-workers<sup>22</sup> demonstrated that CGRP positive fibres exhibited dense ramification and terminal sprouting in the periosteum of fractured rat tibia after seven days. In addition to periosteum, the nerve fibres were found in the middle of the callus interspersed with inflammatory cells and penetrating into secondary minor fractures. Similarly substance P positive fibres are increased during fracture healing<sup>42</sup>. Activation of osteoblastic CGRP receptors result in enhanced osteoblast proliferation *in vitro*<sup>43</sup>. This observation with the finding that CGRP null mice exhibit reduced bone mass due to decreased bone formation rate<sup>44</sup>, suggest that CGRP has a physiological role as a stimulator of bone formation. This hypothesis is further suggested by the highest presence of CGRP positive fibres at site of maximum bone formation in fracture healing<sup>23</sup>. Furthermore, CGRP exerts anti-osteoclastogenic actions by inhibiting osteoclast motility, causing quiescence through a cAMP mechanism<sup>45</sup> and decrease osteoclast formation by interfering with RANKL intracellular pathway<sup>46</sup>. According to our knowledge, no model of fracture healing has investigated the role of VIP-positive nerve fibres. Nevertheless, recent studies have demonstrated that VIP has a protective effect on bone destruction in experimentally-induced arthritis<sup>47</sup>. It has been shown by Mukohyama and co-workers that the negative effects of VIP on bone resorption is mediated through osteoblast/osteoclast cross-talk by decreasing RANK and RANKL expression in osteoclasts and osteoblasts respectively and by increasing osteoblasts expression of osteoprotegerin<sup>48</sup>. Lundberg and co-workers described also that VIP treatment causes a rapid cytoplasmic contraction of osteoclasts along with an associated decrease in motility<sup>49</sup>. Furthermore, VIP decreased levels of IL-1 $\beta$ , IL-6, IL-11, IL-17 and TNF- $\alpha$  involved in the inflammatory process<sup>50</sup>.

The discovery of the cholinergic anti-inflammatory pathway by Tracey and co-workers suggested also a central control of inflammation<sup>51</sup>. Following tissue injury or infection, macrophages respond early by releasing cytokines (TNF- $\alpha$ ; IL-1 $\beta$ ; high mobility group box 1, HMGB1) in their surrounding in order to restore health. If the cytokine response however is unbalanced or excessive, the same mediators can cause disease. The balance of cytokine generates an afferent vagus nerve activity which is integrated by brain networks. The efferent response inhibits cytokine production via pathways dependent on the alpha7 subunit of the acetylcholine receptor ( $\alpha$ 7nAChR) on macrophages and other cells. Activation of the  $\alpha$ 7nAChR results in decreased nuclear translocation of NF- $\kappa$ B, the major transcription factor involved in cytokine production, as well as activation of the transcription factor STAT3 via phosphorylation by JAK2<sup>52</sup>. Recently, van Maanen and co-workers reported that unilateral cervical vagotomy exacerbated collagen-induced arthritis in mice and that treatment with a  $\alpha$ 7nAChR agonist

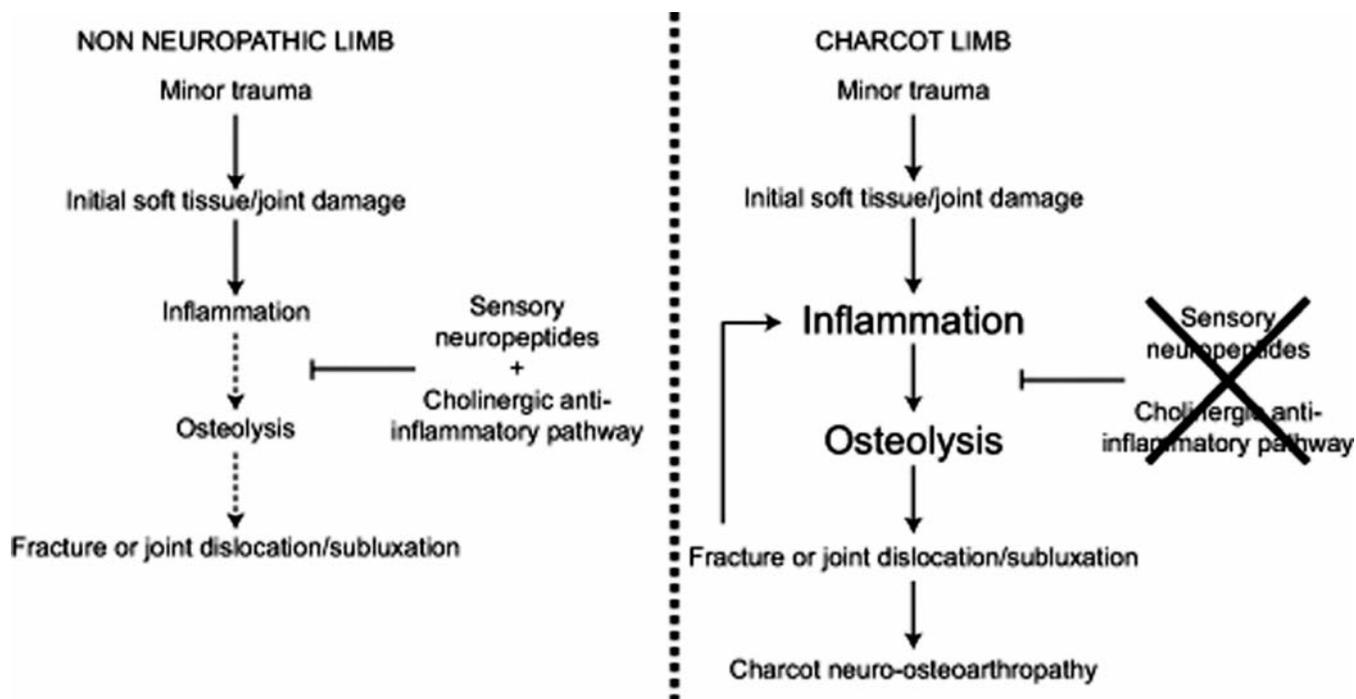
ameliorated the disease<sup>53</sup>, indicating that the cholinergic anti-inflammatory pathway exerts a control on joint inflammation. However, little is known about the involvement of this system in fracture healing, but it is plausible that it play a role in controlling inflammation in soft tissue around the fracture site. In the presence of neuropathy, trauma leading to initial soft tissue and joint damage may result in an exaggerated and uncontrolled inflammatory response which predisposes to multiple fractures and disruption of associated joints.

## Relevance of neurogenic control of bone remodelling and fracture healing in CNO

In diabetes, CNO developed exclusively in patients affected by diabetic neuropathy. Diabetic neuropathy is described histopathologically with axonal degeneration, demyelination, and atrophy, in association with failed axonal regeneration, remyelination, and synaptogenesis<sup>54</sup>. Nerve damage in diabetes can influence large fibres, small fibres are both. Small nerve fibres involvement often occurs early and may be present before objective signs or electrophysiological evidence of large fibre deficits. Small fibre loss is seen first in the lower limbs as pain and hyperalgesia followed by loss of thermal sensitivity, and reduced light touch and pin prick sensation<sup>55</sup>. However, it is still controversial whether large, small or both fibres are responsible for CNO. Certainly in diabetes both large and small fibre neuropathies have been described but the specific importance of only small fibre neuropathy is emphasised in other diseases which can lead to the development of CNO (i.e. syringomyelia, congenital sensory neuropathy and heavy metal poisoning). Donaghy and co-workers reported that in syringomyelia, sensory neuropathy with selective reduction of myelinated nerve fibres resulted in mutilating acropathy<sup>56</sup>. We have also reported previously that diabetic patients with CNO have selective small fibre loss for cold sensation with preservation of warm perception and sensation to light touch<sup>57</sup>. However, in contrast Guy and co-workers demonstrated both small and large nerve fibre loss in their series of CNO<sup>58</sup>.

Although a reduction of substance P and CGRP has been demonstrated in diabetes<sup>59</sup>, their involvement in CNO aetiology has never been proven. Recently, Koeck and co-workers, reported that in CNO compared with joint osteoarthritis, a marked loss of sympathetic nerve fibres were found while sensory substance P-positive nerve fibres were not significantly decreased<sup>60</sup>. However, a reduction of substance P sensory nerve fibres has also been demonstrated in osteoarthritis<sup>61,62</sup> and could explain why in this study the number of substance P-positive fibres was not decreased. Similarly, La Fontaine and co-workers reported that in bone sample of CNO patients, there was a trend for less CGRP positive nerve fibres compared with healthy volunteers although it did not reach statistical significance due to a low number of patients included in that study<sup>63</sup>.

In the acute phase of CNO the foot is swollen and inflamed. It is characterised by bone resorption leading to intra-articular fractures and finally CNO. Inflammation is characterised by the local release of inflammatory mediators such as TNF- $\alpha$ , IL-1 $\beta$



**Figure 2.** Schematic representation of how uncontrolled inflammation may lead to the development of CNO.

in order to recruit leukocytes and initiate a response to the damage tissue. We observed that in CNO, the serum levels of TNF- $\alpha$  were significantly raised compared with diabetic and healthy controls and correlated with the extent of bone resorption (unpublished data) indicating a possible uncontrolled inflammatory response. Reinforcing the idea of uncontrolled inflammation is our previous observations that in acute CNO osteoclasts are more aggressive compared with diabetic and healthy controls and capable of bone resorption even in the absence of any osteoclastic factors<sup>64</sup>. Compared to diabetic and healthy controls, we observed that the number of CD14 positive cells is significantly increased in the blood of acute CNO patients and correlate with the serum levels of TNF- $\alpha$ <sup>65</sup>. CD14 positive cells are a subclass of circulating monocytes which re-enter the damaged tissue upon inflammation. CD14 positive cells are the most potent circulating cells to differentiate into bone-resorbing osteoclasts<sup>66</sup>. It is intriguing to observe that an increase in circulating osteoclast precursors in the blood stream of CNO patients and an aggressive osteoclastic behaviour in this condition do not result in excessive bone resorption in other part of the skeleton than in those affected by neuropathy. Furthermore, osteosclerosis is commonly observed in chronic CNO and it is plausible that neuropathy also lead to an uncontrolled fracture healing. Based on clinical and laboratory observations, we propose that in CNO the inflammatory response to acute or repeated minor trauma is exaggerated and uncontrolled leading to excessive osteolysis which results in multiple fractures and joint dislocation, characteristic of classical Charcot joint (Figure 2). One of the reasons could be the downregulation of inhibitory nerve

pathways (loss of sensory neuropeptide and cholinergic anti-inflammatory pathway) which may result in an exaggerated inflammatory response to minor trauma. As mentioned above, CGRP and VIP are strong inhibitors of osteoclast resorption and it is also plausible that their loss in acute CNO patients could results in excessive and unbalanced bone resorption in response to an unbalanced and excessive inflammation.

There has been little advance in the management of this condition since it was first describe by Charcot in 1868. At present the standard treatment of CNO is casting and immobilisation as indeed it was at the time of Jean-Martin Charcot. Recently, anti-resorptive therapies such as pamidronate, alendronate and calcitonin have been introduced<sup>67-69</sup>. However, the therapeutic response to these agents has been limited and they have not become standard therapy. To develop more successful treatment it is important to understand better the mechanism of CNO and the role of the peripheral nervous system and sensory neuropeptides in local control of inflammation. Currently, it is common belief that the real aetiology lies somewhere in between the neurovascular and neurotraumatic theories. We know that neuropathy is an absolute pre-requisite for the development of the CNO and if the interaction between nerve and musculoskeletal system in the development of the CNO is elucidated, this will bring a new approach to treat this severely disabling condition.

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