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FOOT AND ANKLE

Neuroarthropathy in diabetes: pathogenesis of Charcot arthropathy

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Charcot neuroarthropathy is a rare but serious complication of diabetes, causing progressive destruction of the bones and joints of the foot leading to deformity, altered biomechanics and an increased risk of ulceration.

Management is complicated by a lack of consensus on diagnostic criteria and an incomplete understanding of the pathogenesis. In this review, we consider recent insights into the development of Charcot neuroarthropathy.

It is likely to be dependent on several interrelated factors which may include a genetic pre-disposition in combination with diabetic neuropathy. This leads to decreased neuropeptides (nitric oxide and calcitonin gene-related peptide), which may affect the normal coupling of bone formation and resorption, and increased levels of Receptor activator of nuclear factor kappa-B ligand, potentiating osteoclastogenesis.

Repetitive unrecognized trauma due to neuropathy increases levels of pro-inflammatory cytokines (interleukin-1β, interleukin-6, tumour necrosis factor α) which could also contribute to increased bone resorption, in combination with a pre-inflammatory state, with increased autoimmune reactivity and a profile of monocytes primed to transform into osteoclasts - cluster of differentiation 14 (CD14).

Increased blood glucose and loss of circulating Receptor for Advanced Glycation End-Products (AGLEPs), leading to increased non-enzymatic glycation of collagen and accumulation of AGLEPs in the tissues of the foot, may also contribute to the pathological process.

An understanding of the relative contributions of each of these mechanisms and a final common pathway for the development of Charcot neuroarthropathy are still lacking.

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Article focus

- A synthesis of the current knowledge on the pathways leading to the development of Charcot neuroarthropathy in diabetic neuropathy.

Key messages

- Charcot neuroarthropathy develops due to an interplay of several mechanisms including, abnormalities of bone formation and resorption, upregulation of pre-inflammatory mediators and non-enzymatic glycosylation of tissue.

Strengths and limitations

- A up-to-date summary of the state of knowledge regarding the pathogenesis of Charcot neuroarthropathy.
- A final common pathway for the development of Charcot neuroarthropathy remains elusive.

Introduction

Charcot neuroarthropathy (CNA) is a rare but serious complication of peripheral neuropathy, with fracture and dislocation of the bones and joints of the foot leading to deformity, altered biomechanics and an increased risk of ulceration if left untreated. Diabetes mellitus is the most common cause of neuropathy and consequently of CNA in the developed world. The prevalence of CNA is disputed, but is likely to affect between 1% and 2% of patients with diabetic neuropathy.[1,2] The underlying mechanisms were initially thought to be repeated trauma to the insensate foot (neurotraumatic) combined with dysregulation of the vasomotor and trophic nerve supply (neurovascular).

Management is complicated by a lack of consensus on the diagnostic criteria and an incomplete understanding of the pathogenesis. More recent work has provided an

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insight into the role of inflammation and abnormal bone in the pathogenesis of CNA. However, most studies are small and document the markers of bone turnover and inflammation without conclusive evidence of causation.[3] Few studies include histology of the bone and soft tissues of the feet in patients with CNA. La Fontaine et al[4] investigated the quality of bone in seven normal subjects, eight patients with diabetes and five with active and five with chronic CNA. In the samples from those with diabetes they identified thinner trabeculae, reduced cellularity and thickened tunica media of the vessels in the marrow spaces. The samples from those with CNA had more Howship's lacunae, more woven bone and an inflammatory infiltrate of lymphocytes and eosinophils in the marrow spaces, suggesting that inflammation and reduced bone density are both involved in its pathogenesis.

An understanding of the relative contributions of genetic susceptibility, inflammation, altered neurotrophic factors and disordered bone turnover and a final common pathway for the development of CNA are still lacking. In this paper we review the literature relating to the pathogenesis of CNA at a molecular level.

Search strategy. The search terms 'Charcot foot' and 'neuroarthropathy' were applied to EMBASE (1974 to the present) and MEDLINE (1946 to the present), producing 895 articles. Removal of duplicates resulted in 809 and review of titles for relevance to pathogenesis left 66 articles. Further exclusions were made after review of the abstracts, leaving 45 articles to be reviewed in full. A further eight were then identified from the references of these papers.

Inflammation and the balance of pro-/anti-inflammatory cytokines. In 2016, Molligan et al[5] published a small study of the role of the synovium and fibroblast-like synoviocytes in the development of Charcot neuroarthropathy. Synovial samples were taken from seven feet of non-diabetic control patients undergoing surgery for correction of a deformity or osteoarthritis and seven with CNA, although whether it was active or inactive was not stated. They demonstrated a normal single-layer synovium in the controls, compared with an inflammatory multi-layered synovium with increased vascularity and a lymphocytic infiltrate in the patients with CNA. There was an increased propensity of the synovium to invasive behaviour after the addition of Tumour Necrosis Factor α (TNFα) to the culture, as is found with the synovium in rheumatoid arthritis. They also saw a significant increase in cadherin-11 expression in the CNA synovium. Cadherin-11 is a cell membrane glycoprotein involved in signaling and previously found to be fundamental to the formation of pannus in rheumatoid synovium.[6] Expression of interleukin-6 (IL6), receptor activator of nuclear factor kappa-B ligand (RANKL), A Disintegrin and Metalloproteinase with Thrombospondin Motifs-4 and -5 (ADAMTS4 and ADAMTS5) were all markedly increased in patients with CNA.[5] The ADAMTS family are metalloproteases, which are important in remodelling the extracellular matrix; they are also implicated in the development of osteoarthritis.[7]

Increase in the pro-inflammatory cytokines, TNFα, IL1β and IL6, which promote local inflammation and the maturation and proliferation of osteoclasts via the RANKL pathway, have been documented in CNA.[8,9] The abnormal persistence of the inflammatory response and the inability to terminate the response have been suggested as causative factors in the pathogenesis of CNA.[10] A recent study[11] using peripheral venous blood samples from ten patients with CNA, eight with diabetes and eight controls, showed that monocytes from those with CNA spontaneously produced TNFα, IL1β and IL6, whereas the control patients, with and without diabetes, did not. *Ex vivo* stimulation of cultured monocytes with lipopolysaccharide also significantly increased the release of TNFα, IL1β and IL6 compared with controls and those with diabetes, although significantly lower levels of the anti-inflammatory cytokines IL4 and IL10 were released, indicating that the monocytes of patients with CNA are primed for a more intense inflammatory reaction.[12] The monocytes from the patients with CNA also demonstrated increased resistance to serum withdrawal-induced apoptosis compared with controls. This shows a decreased ability to terminate the inflammatory response, as well as increased intensity of expression of the markers CD40, CD80 and CD86, which are involved in antigen presentation to T-cells and therefore the activation of the RANKL pathway.[9]

Petrova et al[13] linked markers of inflammation with bone turnover in a study of 81 patients, 35 with CNA, 34 with diabetes but without neuropathy and 12 normal controls. Significant differences in C-reactive protein (CRP), TNFα and IL6 were seen between the patients with CNA and the diabetic and control groups. TNFα and IL6 were also found to significantly decrease between presentation and follow-up at three months in those with CNA. Levels of c-terminal telopeptide of collagen and osteoprotegerin were significantly increased in those with CNA and positively correlated with TNFα and IL6 at the time of presentation, linking levels of inflammatory cytokines with the degree of bone resorption in CNA.

Increased extracellular vesicles have been found in the peripheral blood of patients with acute CNA compared with controls. Extracellular vesicles are membrane-bound nanostructures, which may be involved in communication between cells that can transport contents protected from the extracellular matrix. They have been identified at increased levels in cardiovascular and autoimmune diseases and those shed from osteoblasts have been found to contain RANKL. Schara et al[14] have shown levels of extracellular vesicles in CNA to be associated with CRP and increased temperature of the foot and have suggested therefore that they may be useful in monitoring

clinical progression. However, the contents of these vesicles are not known and the mechanisms by which they are associated with the development and progression of CNA have not been studied.

Although it is clear that inflammation is a key component in the development and persistence of CNA, it is not clear what the initiating event of the cascade is, or how the predisposition to a pro-inflammatory phenotype in diabetes interacts with abnormal bone turnover to produce the bony and soft-tissue changes.

RANKL pathway and bone turnover. Low bone mineral density is recognized as a feature of diabetes, particularly type I.[15,16] This is exaggerated in CNA, with disordered bone turnover mediated via the RANKL-NFκB pathway.[17,18] Bone turnover in CNA was investigated by Gough et al (1997),[19] using pyrodinoline-crosslinked carboxy-terminal telopeptide domain of type-I collagen (ICTP) as a marker of bone resorption and carboxy-terminal propeptide of type-I collagen (PICP) and alkaline phosphatase as markers of bone formation.[20] Peripheral venous and dorsal foot venous blood samples were collected from 16 patients with acute CNA, 16 with chronic CNA, ten with diabetes and ten controls. A significant increase in ICTP was found in acute *versus* chronic CNA with a significant correlation between local and systemic levels ($r = 0.986$). No difference was found in the levels of alkaline phosphatase or PICP, suggesting that the pathology of acute CNA is an up-regulation of bone resorption rather than a suppression of bone formation. A small study comparing 23 patients with a fracture pattern of CNA with a 23 who had a dislocation pattern of CNA, found an association with decreased peripheral bone mineral density only in those with the fracture pattern.[21] This introduces the possibility that the type of deformity that develops depends on the pre-existing bone density.

RANKL has been found to be upregulated by several pathological processes in diabetes.[22] Increased ambient glucose has been shown to induce activation of NFκB in a porcine vascular smooth muscle cell model.[23] Oxidative stress, caused by the addition of peroxide to culture, induced NFκB signaling in an *in vitro* rat cell culture model.[24] The presence of Advanced Glycation End-products in a bovine endothelial cell model induced NFκB but the effect was reversed by the addition of antioxidants to the culture.[25] RANKL levels were shown to be increased in the serum of 12 patients with CNA compared with 10 patients with diabetes and five controls, and immunohistochemistry demonstrated upregulation in areas of the tibial artery affected by medial calcification in those with CNA.[26] NFκB was shown to be inhibited by physiological levels of insulin added to human aortic endothelial cells in culture.[27]

A small study by Bergamini et al[28] in 2016 provided potentially conflicting data by demonstrating significantly decreased levels of RANKL in circulating peripheral blood monocytes from patients with acute CNA compared with controls. They found that increased levels of circulating RANKL coincided with clinical resolution and suggested that there is a local compensatory mechanism in acute CNA which limits bone remodelling. Most other studies have included patients with more chronic disease, which may have provided a different profile of RANKL expression.

The Wnt/β-catenin signaling pathway is involved in both bone and vascular metabolism and has also been shown to be disrupted in diabetes, with serum levels of sclerostin and Dickkopf-1 found to be significantly higher in post-menopausal women with type-2 diabetes ($n = 40$) than in gender and age-matched controls ($n = 40$).[29]

Cluster of Differentiation 14 (CD 14) -positive cells have the greatest propensity of the monocytes for transformation into osteoclasts.[30] Mabilleau et al[31] investigated the proportion of circulating CD14-positive cells in 11 patients with CNA, ten with diabetes and six controls. They found a 1.7-fold increase in the percentage of CD 14-positive cells in patients with CNA compared with those with diabetes and a 2.1-fold increase compared with controls. They also identified a 1.7-fold increase in the pro-inflammatory cytokine TNFα in patients with CNA compared with those with diabetes and a 2.2-fold increase compared with controls, with a strong correlation between TNFα levels and the proportion of CD 14-positive cells. These findings suggest that the increased bone resorption seen in CNA may be related to systemic priming with osteoclast precursors and increased circulating pro-inflammatory cytokines, and that the RANKL pathway may not be the only mechanism involved.[32]

A number of interlinked processes appear to be responsible for the altered bone turnover seen in diabetic patients, including disruption of the Wnt/β-catenin pathway, increased circulating osteoclast precursors and upregulation of the RANK/RANKL pathway. The underlying mechanisms have not been fully elucidated but are postulated to include modulation by raised circulating glucose and increased oxidative stress.

Neuropeptides (Calcitonin Gene-Related Peptide (CGRP) and nitric oxide (NO)). The neuropeptides CGRP and substance P are released in a non-synaptic manner from small unmyelinated c-type nerve fibres and small myelinated Aδ fibres. These are found in high concentrations in the periosteum and bone marrow.[33] They have been shown to upregulate osteoblastic genes and downregulate osteoclastic gene expressions such as Tartrate-Resistant Acid Phosphatase (TRAP), cathepsin K and NFκB in a mouse bone stromal cell model,[34] with depletion in a rat model resulting in reduced bone density[35] and the absence of CGRP in a knockout mouse model producing an osteopenic phenotype.[36] Endothelial NO synthase (eNOS) acts via the second messenger NO to regulate osteoclast proliferation[37] and is itself regulated

by CGRP.[38] La Fontaine et al[39] investigated the levels and distribution of CGRP and NO in samples of bone from the feet of four diabetic patients without neuropathy, four diabetic patients with neuropathy and four patients with chronic CNA, using immunohistochemistry and quantitative image analysis. They found a non-significant decrease in CGRP expression (as measured on image area) and a significant decrease in eNOS expression between the patients with diabetes but no neuropathy and those with neuropathy with and without CNA. This is an interesting observation but the number of samples was small.

It is likely that the levels of neuropeptides are altered in the neuropathic foot and this may contribute to altered bone turnover but there is currently insufficient evidence for causation.

Hyperglycaemia and Advanced Glycation End-Products (AGEs)

Receptor for AGEs (RAGE) defence. AGEs are proteins and lipids that have been modified by glycation due to the presence of sugars in a non-enzymatic process. Their formation in diabetes is a result of both hyperglycaemia and oxidative stress. They are found particularly in tissues with low turnover such as cortical bone,[40] and form in many tissues as part of the ageing process, including in non-diabetics, and have also been implicated in pathological processes such as atherosclerosis. AGEs induce apoptosis in mesenchymal cells through combination with RAGE.[41] Although RAGE is constitutively expressed it may be responsible for a RANKL-independent pathway for increased osteoclastogenesis and altered bone quality in diabetes.[42] Circulating RAGE is decreased in diabetes and provides reduced defence against the accumulation of AGEs in tissues, which may impair the mineralization of bone matrix.[43] A 2011 study by Witzke et al[44] of 20 patients with non-acute CNA, 30 with diabetes and 30 normal controls revealed seven-fold lower soluble-RAGE values from peripheral venous blood samples in those with CNA than in the controls, and three-fold lower values in those with diabetes. A positive correlation was seen between soluble-RAGE and calcaneal bone stiffness.

As CNA in the diabetic foot is a condition of weight-bearing joints, the mechanical effect of AGEs on type I collagen may play a role in the development of an abnormal mechanical environment. Previous small molecular dynamics studies of collagen-like peptides have established that non-enzymatic crosslinking to form AGEs results in increased tensile Young's modulus and lateral force-displacement ratios at low strain rates compared with un-crosslinked collagen. This may explain the hardening of tissues seen clinically.[45]

RAGE is present on many cell types of the immune system and is involved in the processes of both acute and chronic inflammation through many signaling cascades, particularly NFκB, activated by ligands including S100/calgranulins and High Mobility Group Box 1. Blockade of RAGE in a mouse model of foot injury was shown to suppress the inflammatory response through downregulation of the NFκB pathway.[46] It has been suggested that in environments of high oxidative stress such as ageing and diabetes, ligands for RAGE may become crosslinked, predisposing to a signaling cascade, which favours the perpetuation of chronic inflammation over rapid ligand clearance and resolution of inflammation,[47] although the mechanism underlying this remains unconfirmed and the threshold beyond which injury occurs has not been identified.

Autoimmunity. Rizzo et al[48] studied the involvement of autoimmunity in order to investigate a further mechanism by which AGEs may potentiate inflammation in CNA. Oxidative stress is increased in diabetes and causes sequential oxidative reactions, which increase levels of AGEs and oxidative post-translational modification of tissue proteins.[49,50] Enzyme-linked immunosorbant assay (ELISA) revealed that increased binding of serum samples to oxidatively modified molecules, particularly type II collagen, was seen in patients with CNA and, to a lesser extent, those with diabetic neuropathy.[48]

Genetic factors. Previous work on susceptibility to osteoporosis has identified several single nucleotide polymorphisms (SNPs) in the gene encoding osteoprotegerin, a decoy receptor for RANKL, which prevents binding to RANK and activation of the NFκB pathway, thereby preventing proliferation of osteoclasts.[51] Pitocco et al[52] investigated the G1181C and T245G SNPs in 59 patients with CNA, 41 with diabetic neuropathy and 103 controls, in Italy, and established a positive correlation of the G allele in both SNPs with CNA. Korzon-Burakowska et al[53] performed similar work involving Polish patients. A total of 54 had CNA, 35 had diabetic neuropathy and there were 95 controls. They found an 8.5-fold increased risk of CNA for the thymine-thymine (TT) polymorphism of the 1217 SNP and an 11.5-fold increase in risk for CNA with TT polymorphism of the 245 SNP. This suggested genotypes associated with an underlying susceptibility to the bone homeostasis mechanism in some individuals. However, they could not demonstrate causation and these studies are small and likely to be underpowered.

Conclusion

The development of CNA is dependent on several interrelated factors. Some small studies have suggested an underlying genetic pre-disposition and others have identified accumulation of AGEs in the tissues of the foot and decreased neuropeptides in neuropathy (NO and CGRP), which may affect the normal coupling of bone formation/resorption, potentiating osteoclastogenesis. Increased levels of pro-inflammatory cytokines (IL1β, IL6, TNFα) and RANKL have been identified, however, their temporal relationship to the development and progression of the condition remains unclear.

The relative contributions of genotype, increased pro-inflammatory cytokines and disordered bone turnover and altered neuropeptide profile have yet to be determined. Few studies have included patients with active CNA, making it difficult to draw conclusions on causation and the temporal relationship of these factors with the development and progression of the condition. A final common pathway for the pathogenesis of CNA is yet to be determined and promising therapeutic targets have yet to be identified.

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