Physiopathology of intratendinous calcific deposition

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Running title: Physiopathology of intratendinous calcific deposition
Abstract
Calcific tendinopathy (CT) is a disorder with calcium deposits in the substance of the tendon, chronic activity-related pain, tenderness, localised edema and various degrees of decreased mobility and motility. It is particularly common in the rotator cuff and supraspinatus tendon, Achilles tendon and patellar tendon. The presence of calcific deposits may worsen the clinical manifestations of tendinopathy with an increase in rupture rate, slower recovery times and a higher frequency of post-operative complications. The aetiopathogenesis of CT is still controversial. Rather than formed by precipitation of inorganic ions, CT seems to be the results of an active cell-mediated process and a localized attempt of the tendon to compensate the original decreased stiffness. Tendon healing includes many sequential processes, and disturbances at different stages of healing may lead to different combinations of histopathological changes, diverting the normal healing processes to an abnormal pathway. Better understanding of the pathogenesis is essential for development of effective treatment modalities and for the improvement of clinical outcomes.

Keywords:
Calcific Tendinophaty, Calcific Deposits, Tendons, Review.
Introduction

Calcific tendinopathy (CT) is a disorder with calcium deposits in the substance of the tendon. It is characterized by chronic activity-related pain, tenderness, localized edema and various degrees of decreased mobility and motility. CT is particularly common in the rotator cuff and supraspinatus tendon, Achilles tendon and patellar tendon. CT of the rotator cuff is common in Caucasian populations, with a reported prevalence of 2.7% to 22%, mostly affecting women between 30 and 50 years. The most frequently involved tendon is the supraspinatus tendon, and in 10% of patients the condition is bilateral (fig. 1) [1]. Calcific insertional tendinopathy of the Achilles tendon manifests in different patients populations, including young athletes and older, sedentary and overweight individuals [2]. Usually, radiographs evidence ossification at the insertion of the Achilles tendon or a spur (fish-hook osteophyte) on the superior portion of the calcaneus. CT in this location is often associated with retrocalcaneal bursitis or Haglund’s deformity. The incidence of insertional tendinopathy of the Achilles tendon is not clear. Incidence varies from 5% to the most common presentation in athletes [2]; calcifications of the main body of the tendon are at best uncommon (Fig. 2). CT of the patellar tendon is rare, and most patients with patellar tendinopathy show no evidence of ossification [3].

The presence of calcific deposits may worsen the clinical manifestations of tendinopathy with an increase in rupture rate, slower recovery times and a higher frequency of post-operative complications [4].

The nomenclature of this condition is confusing, and terms such as calcific periarthritis, periarticular apatite deposition, and calcifying tendinitis have been used [5]. We suggest to use the terms “calcific tendinopathy”, as it underlines the lack of a clear pathogenesis when the process is located in the body of tendon, and “insertional calcific tendinopathy”, if an osteophyte is visible at the site of insertion of the tendon in the bone.

The aetiopathogenesis of CT is largely unknown, especially because it remains difficult to clarify the steps which induce crystal deposition into the tendon. These processes require several months and biopsies of the pathologic RC tendons are obtained only towards the end of the natural history of the condition, when patients are symptomatic [6]. Many different theories have been developed [7,8,9]. Recently, proteins such as Bone Morphogenetic Proteins (BMP) and Transglutaminases (TGs) and the expression of several genes involved in tissue remodeling and bone development have been studied, suggesting that CT may have a precise genetic component [10,11,12].

Most of the current treatments modalities are neither effective nor evidence-based because of our poor understanding on the underlying pathogenesis of CT.
Historical review

Many terms have been used to describe localized deposits of calcium in tendons [13], including calcifying tendinitis, calcific tendinitis, calcified tendinitis, calcareous tendinitis, tendinosis calcarea, calcific periarthritis, periarticular apatite deposit [5, 14]. Some of them emphasise the extra-articular location of the deposit, others mention the nature of the compound found in the calcification or the process that might explain its deposition.

At the beginning of the 1950s, it was clear that local degeneration of the involved tendon precedes the deposition of calcium salts [13], and that there may be a constitutional predisposition.

Sandstrom in 1938 speculated that necrosis of the tendon secondary to local ischemia and vascular changes was the first steps to promote deposition of calcified material [7]. Bishop believed that repetitive minor trauma could induce rupture of the fibers of the supraspinatus tendon, hyaline degeneration and the deposition of calcium in the injured tendon [15]. The hypothesis was later supported by Bosworth and colleagues [8]. Urist and Uhthoff suggested the occurrence of an initial cartilage metaplasia of the tendon, followed by an active multifocal and cell-mediated calcifying process [16,9]. Recently, Mohr and Bilger described the process as beginning with the necrosis of tenocytes with concomitant intracellular accumulation of calcium, often in the form of microspheroliths or psammomas [17].

Histology

The specimens obtained at surgery form RC tendons consist of a gritty mass of sandy material or a toothpaste-like fluid, and the deposits were described as a white amorphous mass composed of many small round or ovoid bodies. Later, X-ray diffraction and infrared spectrometry and others techniques identified the material of calcific deposits as calcium carbonate apatite [18,19,20]. CT studies of patellar tendon instead revealed the three-dimensional structure of calcific deposits, which have a porous structure throughout the tendon [21].

Uhthoff and coworkers hypothesized that a favorable environment permits an active process of cell-mediated calcification, usually followed by spontaneous phagocytic resorption [6]. They describe four stages in the calcifying process of the rotator cuff: precalcific phase, calcific phase, resorptive phase, and repair phase. All may occur concomitantly in the same tendon. The precalcific stage involves fibrocartilaginous metaplasia within the tendon. In the second stage, the formative phase, calcific deposits are formed. This stage is subdivided into three phases: formative, resting, and resorptive. Calcium crystals are deposited primarily in matrix vesicles that coalesce to form large foci of calcification separated by chondrocytes and fibrocartilaginous tissue septae. The resting phase occurs when fibrocollagenous tissue borders the foci of calcification indicating
termination of deposition. The resorptive phase is marked by the appearance of thin-walled vascular channels at the periphery of the deposit. Macrophages and multinucleated giant cells then surround the deposit and phagocytose debris with calcium removal. In this phase, the deposit exhibits a thick, creamy, or toothpaste-like material that is often under pressure. The final stage involves the attempt of the tendon to heal. Nakase et al [22] clarified the nature of the multinucleated cells located near the calcium deposits. These were positive for cathepsin K [22] and osteopontin [23] showing a typical osteoclastis phenotype. Cathepsin K is a protease, it is a member of the peptidase C1 protein family, it is predominantly expressed in osteoclasts, and it is involved in bone remodeling and resorption [24]. Osteopontin is a member of the SIBLING glycoprotein (Small Integrin-Binding Ligand N-linked Glycoprotein) family first identified in 1986 in osteoblasts. It play important roles in many physiological and pathological processes, including wound healing and bone remodeling [25]. Osteopontin has been observed in cells surrounding tendon calcifications, although its role has not been further clarified [26].

The model of insertional Achilles CT have been proposed by Benjamin et al [27], based on a rat model. They suggested that insertional CT can develop by endochondral ossification of fibrocartilage at the enthesis of the Achilles tendon, and it is preceded by vascular invasion. No inflammatory cells or microtears have been identified. The authors believe that the increased surface at the tendon–bone junction may represent an adaptive mechanism to increased mechanical loads.

Lui et al studied the histological features of collagenase-induced patellar tendon ossification in a rat model [21]. Many chondrocyte-like cells and the absence of infiltration of inflammatory cells were observed around the calcific deposits. They found a marked loss of collagen Type I and an increase of collagen Type II and Type X which occurred mainly at the chondrocyte-like cells and their surrounding matrix in the calcific deposits. Collagen type II is typical of cartilage and fibrocartilage, and it is resistant to compressive stresses. Type X collagen is a short chain collagen which has been associated with calcific cartilage and/or the expression of the hypertrophic chondrocyte phenotype. It is a marker of endochondral ossification. The same authors subsequently described an increased expression of collagen type III and a high collagen type III/collagen type I ratio [28]. The increase of collagen Type III coincided with thinner, less organized and weaker tendon. Histological specimens of calcific insertional Achilles tendons tendinopathy showed a greater intensity of staining for collagen Type III than normal tendons [29], and higher than normal expression of collagen types III mRNA was detected high in human Achilles tendinopathy [30]. Chondrocyte markers were also evidenced in the clinical samples of calcific insertional Achilles tendinopathy and in rotator cuff tendinopathy [29,31].
Basic Science

Different hypotheses have been put forward to explain the etiopathogenesis of CT.

Some mineralized deposits in the Achilles and patellar tendons were thought to be formed by a process resembling endochondral ossification, with bone formation and remodeling mediated by population of osteoblasts and osteoclasts [32]. Other authors thought that ectopic bone derives from metaplasia of tendon cells into osteogenic cells [22], but the origin of the cells participating in the process of tendon ossification is unknown.

Mesenchymal stem cells are present in tendon tissues [33]. Human and mouse tendons hold cells with universal stem cell characteristics which could differentiate into chondrocytes and osteoblasts [34]. Rui et al isolated Tendon-Derived Stem Cells (TDSCs) from the flexor tendon and patellar tendon of rats [35,36]. Therefore, they proposed that chondral metaplasia and ectopic ossification may be caused by erroneous differentiation of tendon cells [37].

Which condition or stimulus is able to cause this erroneous differentiation of TDSCs? Many proteins could be involved in tendon degeneration, calcification and rearrangement processes, playing different roles in the various phases of calcification and resorption. Among the possible candidates are Bone Morphogenetic Proteins (BMPs) and transglutaminases (TGs). Recently Zang suggested that BMP-2 mediated effects on human TDSCs may contribute to the formation of calcific deposits in CT [38]. We observed an increased expression of Osteopontin, Cathepsin K and TG2 mRNA in the calcific areas of the supraspinatus tendon as compared to the level observed in the normal tissue [39]. TG2 is ubiquitously expressed and plays a role in a variety of cellular processes, including the crosstalk between macrophages and apoptotic cells, glucose tolerance and other processes. It is also important in maintaining the structural integrity of tendons and it could be involved in tendon repair [40]. The increased expression of Osteopontin and TG2 could thus be compatible with their increased production in the calcific area, probably by osteoclast-like cells involved in the resorptive phase [22].

The mRNA and protein expression of major proteoglycans of extracellular matrix, including decorin, aggrecan, biglycan and fibromodulin and their relationship with ectopic chondrogenesis, ossification and loss of matrix organization is observed in a calcified tendinopathy model [28]. Decorin, aggrecan, biglycan and fibromodulin are Small Leucine Rich Repeated Proteoglycans (SLRPs) which participate in collagen-fibril formation, and their expression patterns are altered in chronic tendinopathy [41]. Decorin is a component of connective tissue, binds to type I collagen fibrils, and plays a role in matrix assembly [42]. It is the most abundant SLRP found in tendon mid-substance. Aggrecan and biglycan are common in the fibrocartilaginous regions of the enthesis. Aggrecan has an important role in the adaptation to compressive loads. Fibromodulin participates in
the assembly of the extracellular matrix, and it interacts with type I and type II collagen fibrils [43]. A sustained or increased expression of decorin, aggrecan, biglycan and fibromodulin was found in this calcified tendinopathy model [28], and the presence of ectopic calcification in Achilles, patellar and quadriceps tendons was reported in biglycan and fibromodulin single knock-out mice [44]. Another important feature of SLRPs is their ability to modulate the activity of the resident cell population by binding and sequestering growth factors [45,46]. The differentiation of tendon progenitor cells into chondrocytes and bone cells was modulated by the expression of biglycan and fibromodulin [47]. In normal tendon healing, TDSCs would proliferate and differentiate into tenocytes but, in particular conditions, they would differentiate into chondrocytes or osteoblasts, causing the deposition of the “wrong” extracellular matrix and calcific deposits, resulting in failed healing and pain. The mechanism leading to the erroneous differentiation of TDSCs is not clear. It could be modulated by the expression of biglycan and fibromodulin, and by the expression of chondro-ostogenetic BMPs, such as BMP-2, BMP-4 and BMP-7, which were over-expressed in CT models [48]. Rui et al therefore hypothesized that the erroneous differentiation of TDSCs into chondrocytes or osteoblasts instead of tenocytes could be the pathogenetic mechanism of calcifying tendinopathy [37]. Aberrant differentiation of stem cells have been postulated to be the cause of other disorders, including vascular calcification [49], skin calcification [50] and skeletal calcification [51].

An association between CT and diabetes and thyroid disorders has been proved, but the precise mechanism is still unknown. [1] More than 30% of patients with insulin-dependent diabetes have tendon calcification, and they are more likely to develop asymptomatic deposits [52]. Patients with associated endocrine disorders present earlier onset of the symptoms, longer natural history and they undergo surgery more frequently compared to a control population [53].

A familial predisposition and inherited genetic components has also been postulated as a cause of CT in some instances [54,55,56,57]. Variants within COL5A1 [58], Tenascin C [59] and Matrix Metalloproteinase 3 (MMP3) gene [60] are associated with increased risk of Achilles tendon injuries. Therefore some genetic variants could modify the susceptibility of tendons to matrix degeneration observed in tendinopathy [61].

Non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids injections are commonly prescribed in painful tendinopathy. Some Authors proposed NSAIDs, corticosteroids and some antibiotics as possible factors which may adversely affect tendon healing and which could take part in the process of CT. NSAIDs could modulate tendon cell proliferation [62,63], the expression of extracellular matrix components [64] and degradative enzymes in cells culture studies [37]. Corticosteroids can induce a fibrocartilaginous phenotype in tendon cells [64], and in particular
Dexamethasone could induce human spinal ligament derived cells towards osteogenic differentiation [65]. Fluoroquinolones activate metalloproteinases in tenocytes, leading to collagenolytic injuries [66]. It is therefore possible that NSAID and corticosteroids, which are currently widely used for sports related injuries and for the management of tendinopathy, may influence tenocytes differentiation and proliferation, to affect the course of tendon healing and the development of tendinopathy [67].
Discussion

The aetiopathogenesis of CT is still controversial, especially because it remains difficult to clarify the first steps causing this condition and involved in the development of calcifications (fig.3). Few investigations have been performed on the role of the types of carbonated apatite although the they have been reported to be a single component in the calcific deposits [68]. Gartner et al. [69] observed that the macroscopic differences of calcific deposits was not reflected in the mineralogical structure, and neither chemical compositional change nor a change in the crystal lattice was observed. They stated that no chemical dissolution process of the inorganic material was responsible for the resorption activity in the acute phase. More recently a correlation between the morphology of calcific deposits, different types of carbonated apatite, progressive calcification process and clinical symptoms was postulated [70].

Recent studies suggest that, rather than formed by precipitation of inorganic ions, CT results from an active cell-mediated process. Although there are signs of matrix degeneration in the affected tendons, the deposition of calcium salts may represent a failure attempt of the tendon to heal, and the cells in the healing tendon may play a role in the development of chronic tendinopathy and CT [44,71]. Other studies also reported the presence of resident progenitor cells with multidifferentiation potential in the tendon [33,34,47]. Injection of recombinant human bone morphogenetic protein-2 (rhBMP-2) [72] into the tendon increased ectopic bone formation, indicating that the tendon consisted of cells that were responsive to BMP [38] and were capable of differentiating along the chondro-osseous pathway.

Many different factors such as acute injury, repetitive micro-trauma, and chemical-induced injury may cause damage to the tendon and start the natural healing process. Tendon healing includes many sequential processes such as matrix synthesis and remodeling, neovascularization, neural modulations, recruitment of healing cells, multipotent cells, TDSCs, proliferation, apoptosis. Disturbances at different stages of healing may lead to different combinations of histopathological changes. The normal healing processes are then diverted to an abnormal pathway (Fig.4). Clinical features such as chronic pain, swelling, functional limitations and tendon ruptures are the consequences. Conservative treatments such as NSAIDs or corticosteroids are often prescribed and may further influence the pathways of the failed healing.

Since ossified tendons will have increased stiffness, ossification can be seen as a localized attempt to compensate the original decreased stiffness of the weak tendon. It is possible that the erroneous differentiation of tendon progenitor cells into chondrocytes or osteoblasts instead of tenocytes may contribute to the pathogenesis of CT. The mechanism leading to the erroneous differentiation of TDSCs is not completely understood. Probably, the expression of BMPs,
biglycan, fibromodulin, NSAIDs or corticosteroids, and an unfavorable micro-environment induced by overuse modify the natural healing process of the tendon.

**Conclusions and future perspectives**

We know little about the pathogenesis of CT and many questions remain still unsorted.

Calcium carbonate apatite appears the only component of calcific deposits, but inorganic component of Achilles and patellar CT has been less investigated than the RC. Histological and radiological studies show that the three-dimensional structure of calcific deposits is quite different because they look like a toothpaste-like fluid in the RC while they have a porous structure throughout the tendon in Achilles and patellar tendon [21]. Therefore, we can hypothesise that also the mineralogical structure could have some differences.

“Calcific tendinopathy” and “insertional calcific tendinopathy” are caused by two distinct pathogenetic mechanisms. This is a result of the fact that in the RC tendons calcium carbonate apatite is deposited at first into vesicles which seem to be acellular, and then macrophages and multinucleated giant cells with a typical osteoclastis phenotype [73] surround the deposit and phagocytise debris with calcium removal, producing a toothpaste-like material. This process has not been observed in other tendons (Table 1). This contrasts the study by Gohr et al [74] that isolated calcium vesicles were present in the mature porcine patellar tendon. In that study, however, the enthesis was removed and the central portion of the patellar tendon was used for this study. So his model is more similar to a calcific tendinopathy model of the main body of the tendon than to an insertional CT model.

The mechanism of insertional calcific tendinopathy has been clarified by Benjamin and co-workers, and essentially worldwide accepted [27].

Calcific tendinopathy of the rotator cuff has been investigated with histological studies of specimens obtained from human biopsies, while the CT of Achilles and patellar tendons are based on animal models of collagenase-induced tendinopathy. Therefore, we do not know whether the pathogenesis of calcific tendinopathy of the rotator cuff can be compare to calcific tendinopathy of the Achilles and patellar tendons. Moreover, no pathogenetic studies on the rotator cuff have been published since the late 1970s, and no animal CT studies are present in literature [75]. We do not know why the calcific deposits of the rotator cuff involve the main body of the tendon, while the most common presentation of CT in the Achilles tendon is insertional. Animal models of CT of RC seems to be necessary to understand its pathogenesis. Then no histological and epidemiological studies on CT of the main body of Achilles tendon are published.
Furthermore no clinical or imaging classification has been published in the literature, except for the CT of RC [1].

Also, it is not clear whether the gross morphological anatomy of tendons (for example, the RC is a flat tendon, while the Achilles tendon is cylindrical) plays a role.

The involvement of MSC in the pathogenesis of the CT process [37] and the role of autologous growth factors have been postulated but not clarified [45,46].

While emerging data seems indicate an association between tendinopathies and endocrine disorders such as diabetes, hypercholesterolemia, hypertriglyceridemia, thyroid disorders, estrogen levels alterations [76], the association with CT is unclear, and no physiopathological investigations have been performed.

Little is known about the pathogenesis, and consequently few evidence based therapies are available. Better understanding of the pathogenesis is essential for development of effective treatment modalities and for the improvement of clinical outcomes.
List of abbreviation
CT: Calcific tendinopathy
BMPs: Bone Morphogenetic Proteins
TGs: Transglutaminases
TDSCs: Tendon-Derived Stem Cells
SIBLING: Small Integrin-Binding Ligand N-linked Glycoprotein
SLRPs: Small Leucine Rich Repeated Proteoglycans
MMP3: Metalloproteinase 3
NSAIDs: Non-Steroidal Anti-Inflammatory Drugs
rhBMP-2: Recombinant Human Bone Morphogenetic Protein-2

Competing interests
The Authors declare that they have no competing interests.

Author’s contributions
FO and AGV drafted the initial manuscript and reviewed the literature. NM commented and supervised the manuscript. All Authors read and approved the final manuscript.

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References


Figures Legends

Fig.1: Bilateral CT of the shoulder.
Fig.2: Insertional CT of the Achilles tendon associated with CT of the main body of the tendon.
Fig.3: CT of the subscapularis tendon in a 13 years old boy.
Fig.4: CT of patellar tendon after tendon reconstruction at 1 year follow-up.

Table 1: Pathogenetic models proposed for calcific tendinopathy of RC and insertional calcific tendinopathy of Achilles tendon.
Tab.1

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