Author’s response to reviews

Title: Physiopathology of intratendinous calcific deposition

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Author’s response to reviews: see over
Dear Dr Lin,

Physiopathology of intratendinous calcific deposition

Francesco Oliva, Alessio Giai Via, Nicola Maffulli

Many thanks for the opportunity to revise the above manuscript. Please find attached the revised version of the above manuscript. The comments of the reviewers have been carefully considered, and implemented as follows:

Reviewer 1 – Ann Rosenthal

Introduction

- It is poorly organized and the introduction, for example, could be shortened so that it just includes the first paragraph.

We change as required.

- Reviewer 1 notes that it is important to emphasize early on in this work that the large calcifications detectable on x-ray may only reflect a small percentage of the clinical incidences of calcification in tendon.

We now state Microscopic calcifications which are not detectable at plain radiography can also occur in chronic tendinopathy. A histological study showed high incidence of small calcium deposits in tendinopathic supraspinatus tendons [8]. Microscopic calcium deposits are frequent also in diabetic patients

A section called clinical manifestations could include some of the information in the introduction as well as the ramifications of this process for prognosis.

We changed this section as indicated below:

Clinical manifestation

Clinical manifestations of the calcific process within the tendons include chronic activity-related pain, tenderness, localized edema and various degrees of decreased range of motion (ROM). CT of the rotator cuff shows a tendency toward spontaneous resorption of the deposits and symptoms often resolve spontaneously, although some authors described persistent pain at long time follow-up and persistent reduction of ROM [5,6]. Osteolysis of the greater tuberosity is an uncommon and distinctive form of CT of the shoulder, it is associated with significantly poorer clinical and functional outcome both before and after surgical treatment [7]. Microscopic calcifications which are not detectable at plain radiography can also occur in chronic tendinopathy. A histological study...
showed high incidence of small calcium deposits in tendinopathic supraspinatus tendons [8]. Microscopic calcium deposits are frequent also in diabetic patients [9].

The clinical evolution of insertional CT has been less investigated. Although clinical experience suggests that pain seems improves in older patients even if insertional calcification persists. Generally, the presence of calcific deposits worsens the clinical manifestations of tendinopathy with an increase in rupture rate, slower recovery times and a higher frequency of post-operative complications [10].

- *The division of the paper into sections called "histology" and "basic science" is not clear, and it would be easier for the reader to understand if this part of the manuscript was divided into different theories of pathogenesis, e.g dystrophic calcification, chondrocyte metaplasia, etc*

We change the sections into theories of pathogenesis as required, and we included the subsections called “Reactive calcification, Endochondral ossification, Chondral metaplasia and Predisposing factors”.

We also add a table which summarized the different theories (Table 2)

<table>
<thead>
<tr>
<th>Degenerative calcification</th>
<th>Vascular ischemia</th>
<th>Sandstrom $^{11}$</th>
<th>1938</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repetitive trauma</td>
<td>Bishop$^{12}$ and Bosworth$^{13}$</td>
<td>1939, 1941</td>
<td></td>
</tr>
<tr>
<td>Necrosis of tenocytes and intracellular calcium accumulation</td>
<td>Mohr and Bilger$^{16}$</td>
<td>1990</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reactive calcification</th>
<th>Active cell mediated process</th>
<th>Uhthoff $^{27}$</th>
<th>1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endochondral ossification</td>
<td>Endochondral ossification of fibrocartilage at the enthesis of the tendon</td>
<td>Benjamin $^{33}$</td>
<td>2000</td>
</tr>
<tr>
<td>Chondral metaplasia</td>
<td>Erroneus differentiation of TDSCs</td>
<td>Rui $^{45}$</td>
<td>2011</td>
</tr>
</tbody>
</table>


Thank you for your helpful suggestion. We discuss the article in the text, and we added it to the references.

“The exposure of proteins to high levels of sugar moieties cause the glycosylation of several extracellular matrix proteins, which can modify extracellular matrix by cross-linking proteins. In an
animal study, tenocytes obtained from porcine patellar tendon have been incubated with Glycated type I Collagen, and it was found to be able to increase Tg activity (Rosenthal AK, Gohr CM, Mitton E, Monnier V, Burner T. Advanced glycation end products increase transglutaminase activity in primary porcine tenocytes. J Investig Med 2009, 57:460-466). The author so concluded that the increased production of Tg cross-links could be an additional, novel pathway mediating pathologic changes and that could contribute to the calcific pathogenesis in diabetic tendons”.

- The section on SLRPs contains contradictory material: You say in one sentence that SLRPs are increased in calcific tendinitis and in the same sentence that knocking out these proteoglycans produced exuberant calcification.

We changed:

“A sustained or increased expression of decorin, aggrecan, biglycan and fibromodulin was found in this calcified tendinopathy model [36]. On the other hand the presence of ectopic calcification in Achilles, patellar and quadriceps tendons was also reported in biglycan and fibromodulin knock-out mice [53]”.

For this reason, the role do biglycan and fibromodulin in the pathogenesis of CT is still unclear.
Reviewer 2 – Geraldine McCarthy

- In the first instance, the authors have published a review recently (Sports Med Arthrosc. 2011 Sep;19(3):237-43. Calcific tendinopathy of the rotator cuff tendons. Oliva F, Via AG, Maffulli N.) I do not have access to the full text. I would like to know why the current review represents an advance on the review published in September 2011 on the same subject.

The manuscript “Calcific Tendinopathy of the Rotator Cuff Tendons” is focused only the condition affecting the rotator cuff. In the present manuscript, we consider calcific processes of all tendons, trying to clarify the complexity of the wide range of pathological manifestations of the condition. For example, we discuss about the differences between insertional calcific tendinopathy and calcific tendinopathy of the main body of a tendon.

Abstract

- Refers to decreased mobility and motility – are they not the same thing?

We used both the above words to explain that in some joints such as the shoulder, when CT takes place, patients can lose active movement secondary to pain, and passive movement because of an adhesive capsulitis. We agree that it may be clearer for the readers to use “decreased range of motion”. We have done so in the text

- Suggest remove ‘Rather than formed by precipitation of inorganic ions ‘

Done

- Introduction: should state purpose of the review.

Done. See reviewer 3.

- Authors state (referring to ref 6 from 1976) that process of crystal deposition takes several months – this is speculation and should be so stated.

We deleted the sentence.

- Also, RC tendons needs to be defined viz. rotator cuff

We use only rotator cuff tendons (RCTs)

- Contribution of BMP and TGs are mentioned in the same sentence as genes. These should be separated as this is confusing.

Done.
Histology

- First sentence: typo, ‘form RC’ tendons – should be from.

Done

- The authors discuss the work of Uhthoff et al including the relationship to matrix vesicles. The work by Gohr et al is referred to much later in the manuscript (ref 75) but could be introduced at this point as it is highly relevant to the discussion of the formative phase. It would be worth referring to the work of Shon et al. (Shon W, Folpe AL. Tenosynovitis with psammomatous calcification: a poorly recognized pseudotumor related to repetitive tendinous injury. Am J Surg Pathol. 2010 Jun;34(6):892-5) where an unusual variant of calcific tendonitis, which the authors propose as a distinct entity, is described in detail.

The work of Shon et al describes a distinct and interesting variant of calcific deposition. It involves the tendon but also the tendon sheath and peritendinous soft tissue. But as it is a distinct entity, different from CT, we think it is beyond the purpose of our work.

- Chiou et al have recently shown correlation of variations in morphology of deposited crystals with clinical findings. (Rheumatology (Oxford). 2010 Mar;49(3):548-55. Epub 2009 Dec 23. Correlations among mineral components, progressive calcification process and clinical symptoms of calcific tendonitis Chiou HJ, Hung SC, Lin SY, Wei YS, Li MJ). The authors refer to this in passing giving more emphasis to older studies. The more recent work should be emphasized. (Major revision)

We discuss the article in greater detail as you suggested:

Few investigations have been performed on the role of the types of carbonated apatite, although they have been reported to be a single component in the calcific deposits [21]. Two different types of carbonate apatite compose the calcific deposits, according to the position which carbonate ions (CO$_3^{2-}$) occupy in the hydroxyapatite (HAP). They are defined as A-Type carbonate apatite and B-Type Carbonate apatite [22]. Gartner et al [23] observed that the macroscopic differences of calcific deposits were 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 Chiou et al studied the chemical components in CT of the RCTs, and they observed that different quantities of A- and B-type carbonated apatite changed in the formative, resting and resorption phases [24]. They noted reduced amounts A-Type carbonated apatite and increased amounts located in the B positions during the process of progressive calcification. The same Authors classified calcific depositions into four shapes according to
ultrasonographic findings: arc shape, fragmented or punctuated shape, nodular shape and cystic shape. They also found a correlation between the morphology of the calcified deposits and clinical symptoms of the affected shoulder (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Calcific stage</th>
<th>Morphologic shape of calcic deposition</th>
<th>Clinical symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-calcific stage</td>
<td>The correlation was not evaluated</td>
<td></td>
</tr>
<tr>
<td>Calcific stage</td>
<td>Formative phase</td>
<td>Arc or fragmented/punctuated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resting phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resorpive phase</td>
</tr>
<tr>
<td>Post-calcific stage</td>
<td>The correlation was not evaluated</td>
<td></td>
</tr>
</tbody>
</table>

- Reviewer 2 suggests that if there is evidence that these drugs produce calcific tendonitis, to please include these references.

To our knowledge, there is evidence that NSAIDs and corticosteroids can adversely affect tendon healing by perpetuating degenerative process. There is also some evidence that Dexamethasone could induce osteoblastic differentiation of human spinal ligament cells (Murata H, Tanaka H, Taguchi T, Shiigi E, Mizokami H, Sugiyama T, Kawai S: Dexamethasone induces human spinal ligament derived cells toward osteogenic differentiation. J Cell Biochem 2004). We discuss it into the Discussion.

Conclusion

- The authors state that apatite is ‘deposited at first into matrix vesicles which seem to be acellular’. This statement does not reflect the potential active role of the matrix vesicle in the mineralization process. This role has been noted in mineralization of cartilage also. (major revision)

We discuss the role of matrix vesicles into the section mineral components of the article.

“Recently, matrix vesicles have been isolated in mature porcine patellar tendons [25]. Matrix vesicles are small extracellular organelles which are involved in mineralization of the extracellular matrix in many tissues, including bone and cartilage [26]. Previous studies demonstrate the presence of matrix vesicles near calcific deposition of the RCTs [16-27], and recently they have
been isolated also in the extracellular matrix of normal patellar tendon [25]. The authors pointed out the importance of extracellular matrix vesicles in pathogenetic mechanism of CT. In the normal matrix, the vesicles are inhibited from mineralizing, but in pathological conditions, such as injuries or matrix degeneration secondary to age or diabetes, they may be permitted to mineralize.

We think that they could be involved in the process of calcific tendinopathy of the main body of the tendon, but their role is not clear in calcific insertional tendinopathy, as it seems a different process (Benjamin).

- Figures 3 and 4 do not hugely add to the statements to which they are linked.

We delete fig. 4.

Fig. 3 shows a CT of the subscapularis tendon in a 13 years old boy. We used this figure because, even though degenerative changes may represent an important stimulus to calcific deposition into the RCTs, they can not be solely responsible. In fact we are not able to explain the deposition of calcium salt in the RCTs of a young boy only with a reactive degenerative theory. Probably, genetic components may play a role.

We change:

“A familial predisposition and inherited genetic components has also been postulated as a cause of CT in some circumstances [64,65,66,67]. Variants within COL5A1 [67], Tenascin C [69] and Matrix Metalloproteinase 3 (MMP3) gene [70] are associated with increased risk of Achilles tendon injuries. CT of the RCTs have been observed in children and it could not be related to degenerative changes [71] (fig.3). Therefore, some genetic variants can modify the susceptibility of tendons to matrix degeneration observed in tendinopathy [72]”.
Reviewer 3 – Lin Lee

Introduction

• Introduction: Should state purpose of review

Done:

Most of the current treatment modalities are neither effective nor evidence-based because of our poor understanding on the underlying pathogenesis of CT. We review the present knowledge on this topic to stimulate further research.

Discussion

• This section is a summing up of what was previously discussed in the section before. In order to ensure the review is not too repetitive, you might want to consider shortening this part to make the take home message clearer.

Done:


• Currently, your Figure 4 is a radiograph. This is interesting, but it might be more informative to perhaps have instead a simple schematic to outline the abnormal pathway that you have mentioned here.

Done. We think however that it is difficult to portray the calcific process in a simple schematic because different process may take part in the pathogenesis of CT and they seem not to be mutually exclusive.

• Reviewer 2 notes that the work by Gohr et al should actually be discussed earlier in the manuscript, although you could refer to it again here in the conclusions.

Done

References

• Please consider adding and discussing within your review, the following reference, as recommended by Reviewer 1.

Done. We discuss within the text and add to the references the article suggest by Reviewer 1 “Rosenthal AK, Gohr CM, Mitton E, Monnier V, Burner T. Advanced glycation end products

Finally, we would like to elucidate that the surname of the second author is “Giai Via”, and the first name is “Alessio”.

We thank the Editorial Board for having given us the opportunity to revise our manuscript. We appreciate yours and the reviewer's comments. I hope that the additions have now improved the manuscript, and that it has now reached the standard necessary to be formally accepted for publication.

Yours sincerely,

Nicola Maffulli