



Article

Calcific Tendinitis Is Mainly Associated with Basic Calcium Phosphate Deposition, but Rare Cases of Calcium Pyrophosphate Can Occur

Jannes Anton Knop ¹, Andrea Schwab ¹ , Nicole Märtens ¹, Christian Wybrandski ², Sina Stücker ³ , Agnieszka Halm-Pozniak ¹ , Alexander Berth ¹ , Christoph H. Lohmann ¹ and Jessica Bertrand ^{1,*}

¹ Department of Orthopedic Surgery, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany; andrea.schwab@med.ovgu.de (A.S.); nicole.maertens@gmx.de (N.M.); agnieszka.halm-pozniak@med.ovgu.de (A.H.-P.); alexander.berth@med.ovgu.de (A.B.); christoph.lohmann@med.ovgu.de (C.H.L.)

² Department of Radiology and Nuclear Medicine, University Hospital Magdeburg, 39120 Magdeburg, Germany; christian.wybrandski@gmail.com

³ Institute of Physiological Chemistry and Pathobiochemistry, Waldeyerstraße 15, 48149 Münster, Germany; sinastck@gmail.com

* Correspondence: jessica.bertrand@med.ovgu.de

Abstract

Calcific tendinitis (CT) is a condition that most commonly affects the rotator cuff tendons of the shoulder joint. Fundamental crystal morphology and composition underlying different radiological stages of CT remained insufficiently characterized. We collected human tendon samples from 33 patients with CT and 12 control patients. CT patients exhibited an increased incidence of comorbidities, especially high blood pressure and disorders of fat metabolism. Based on the Gärtner classification, calcific deposits were classified as type I in two patients, type II in 14 patients, and type III in 17 patients. Tendon calcification of the controls was significantly lower compared to the CT group. No differences in the amount of histological calcification between the Gärtner groups were observed. A correlation between the radiological size of calcification and the amount of calcification detected in histology was found. SEM-EDS and Raman identified basic calcium phosphate to be the predominant crystal type in tendons from CT patients, with only two calcium pyrophosphate detections. CT is mainly associated with BCP deposition. Calcified depot size was associated with pain intensity, while diabetes mellitus was observed more frequently in CT patients than in controls. BCP may be a focus of future mechanistic studies on CT.

Keywords: calcification; tendon; calcific tendinitis; basic calcium phosphate; calcium pyrophosphate



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1. Introduction

CT is a condition that most commonly affects the rotator cuff tendons of the shoulder joint. It is characterized by calcium-containing deposits in the tendons, which can cause pain, inflammation and mobility restriction in the affected shoulder [1,2]. CT is the cause of subacromial shoulder pain in up to 42.5% of cases and it is a common cause of shoulder-related incapacity to work [3]. However, the mechanisms that lead to crystal deposition in CT remain unclear. An association between CT and metabolic disorders such as diabetes mellitus or hypothyroidism has been described [4,5].

Calcification can be physiological, as in the case of bone formation, or pathological, as in the case of ectopic calcification. Ectopic calcification is primarily a result of BCP crystal deposition [6]. In rare cases, calcium may also be deposited as CPP [7]. A study of patients with CT in the shoulder, wrist, hand, hip or knee joints showed that the calcium deposits contained BCP [8]. Another study of acute and chronic shoulder calcific deposits also showed that deposits in either case were composed of BCP [9]. CPP has rarely been found in CT patients so far [10–13].

The Gärtner classification is used to describe the marginal sharpness and structure of calcified deposits on radiographic images, which is associated with the spontaneous course of CT. According to Gärtner, type I calcific deposits are characterized by their sharp and well-defined border with a dense structure, type II deposits either appear with a sharp border and an inhomogeneous structure or show a vague border with a homogenous structure. Type III calcific deposits have a vague border with a cloudy appearance and are transparent in structure. The Gärtner classification allows pathogenetic conclusions to be drawn about the onset of the resorption [14,15].

CT is a cell-mediated process consisting of precalcific, calcific, and postcalcific stages. The calcific stage can further be subdivided into formative, resting, and resorptive phases. During the formative phase, calcium-containing crystals are deposited and form large foci of calcification. Fibro-collagenous tissue borders are characteristic of the resting phase and indicate the termination of deposits at this site. During the resorptive phase they are spontaneously resorbed often forming a creamy or toothpaste-like material in the rotator cuff. At the postcalcific stage, the space of resorbing calcific deposits is remodeled, characterized by the formation of collagen type I and signs of collagen alignment along the tendon axis [15].

Radiologically, periarticular shoulder calcifications can be classified as nodular, arc-shaped, fragmented/punctate, or cystic [14,16,17]. Arc and fragmented/punctuate shapes were associated with the formative phase and mild pain intensity, whereas nodular and cystic shapes, both associated with the resorptive phase, were associated with moderate to severe pain. The calcified deposits in the formative phase were found to consist of poorly crystalline, non-stoichiometric apatite. However, the progression of calcification through the resting and resorption phases was found to result in a shift towards mature, crystalline, stoichiometric hydroxyapatite, accompanied by changes in the type and degree of carbonate substitution [16].

Despite radiological and histological classifications, the fundamental crystal composition has not been adequately characterized in human tendon tissue using combined clinical, radiological, histological, and spectroscopic approaches.

In this study, we collected the clinical parameters of CT patients. Radiological staging was complemented by volumetric and histological assessment. The samples were further analyzed by SEM-EDS imaging and Raman spectroscopy, which enables reliable molecular-level discrimination between different calcification types. A correlation between patient characteristics and a specific calcium crystal identification using high-resolution imaging has not been performed before.

2. Materials and Methods

2.1. Patient Characteristics

A total of 45 patients were included in the study, divided into 33 patients with CT and 12 control patients (Table 1). The CT cohort showed a balanced sex distribution, whereas more males than females were included in the control cohort. Patients in the control group were on average 40 years old, while CT patients were on average 57 years old. The BMI was slightly lower in the CT cohort at 27 compared to 29 in the control patients.

Table 1. Demographic data of the patient cohort (n = 45) characterized in this study. M. supraspinatus (SSP), M. infraspinatus (ISP), M. subscapularis (SSC).

Cohort	n	Sex	Age [yr]	BMI	Location	Bursitis
Calcific tendinitis	33	♂17 ♀16	57 ± 9.1	26.6 ± 5	SSP: 30 ISP: 3	24
Control samples	12	♂10 ♀2	40.5 ± 14.0	29.3 ± 3.8	SSC: 9 SSP: 3	2

Tissue samples were taken from the tendons of the rotator cuff muscles: M. supraspinatus (SSP), M. infraspinatus (ISP) and M. subscapularis (SSC). Tendon sampling was possible during stabilizing Latarjet procedures on the shoulder joint in control patients without CT. Bursitis was present in 24 CT patients, but only in two control patients.

2.2. Surgery and Tendon Sampling

The study was approved by the Institutional Review Board (IRB) of the Medical School at Otto-von-Guericke University Magdeburg (84/19). Calcified tendon material from 33 CT patients was harvested arthroscopically during surgery in the Orthopedic Department under general anesthesia in the standard beach-chair position. After localizing the calcification deposit in the rotator cuff tendon in the subacromial zone, the sample was collected via a lateral portal using biopsy forceps. Tendon samples without CT or macroscopic signs of calcification from 12 patients were used as controls. The majority of control samples (8/12) were obtained from patients undergoing an open Latarjet procedure due to chronic shoulder instability. The remaining samples were harvested during other shoulder surgeries, including procedures for rotator cuff pathology and shoulder arthroplasty. Baseline data, including age, sex, symptom duration, localization within the rotator cuff, comorbidities, and previous treatment for calcific tendinitis were collected and used for data analysis. Passive range of motion (ROM), including forward elevation (FE), retroversion (RV), abduction (ABD), adduction (ADD), internal rotation (IR), and external rotation (ER), was measured to evaluate the preoperative functional status of the shoulder joint. The visual analog scale (VAS) was used to assess the subjective pain at rest and during active motion, with 0 indicating no pain and 10 indicating extreme pain.

2.3. Radiological Measurement of Calcification

Radiography was used to diagnose calcific tendinitis. Plain shoulder radiography was performed in anteroposterior, axillary, lateral and outlet views. Based on plain radiographic images we grouped the CT positive patients and the control group according to the Gartner classification into the three types (type I, II and III) [14].

In addition, the calcification deposits were measured using fat-suppressed proton density weighted images (fsPDWI) and T2-weighted images (T2WI) in the oblique coronal, oblique sagittal and transverse planes on a 1.0T Panorama HFO scanner (Philips Healthcare, Best, The Netherlands). Given the slice thickness of the imaging data (2.5–4 mm), we performed an elliptical approximation of the volume using voxel-based calculations.

2.4. Scanning Electron Microscopy and EDS Analysis

Tendon samples were analyzed using scanning electron microscopy (SEM). We employed a FEI XL30 Scanning Electron Microscope (FEI Scios DualBeam equipped with an EDAX EDS system, Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with a cold field emission cathode and an EDAX EDS system. It allows a voltage range from 0.5 to 30 kV and a resolution of 1 nm at 15 kV with a working distance (WD) of 11.9 mm in our measurements. The investigations were conducted with an acceleration voltage of

7.5 kV and magnifications from 3500 to 25,000 $\times$. In preparation for EDS analysis chrome sputtering was performed.

2.5. Raman Spectroscopy

Calcium-containing crystals were identified by confocal Raman spectroscopy (Bruker Senterra II, OPUS 7.8, Bruker, Ettlingen, Germany) as described by Buchholz and colleagues [18]. Tendon sections were deparaffinized in xylene and air-dried. Raman spectroscopy was performed with a 785 nm laser of 100 mW power, an integration time of 5 s, a spectral resolution of 4 cm⁻¹, 10 coadditions, and an aperture of 25 $\times$ 1000. Raman spectra were cut to the area of interest (300–1800 cm⁻¹) and baseline corrected to minimize background and autofluorescence signals. The tissue section was screened by multiple point analyses using the crystal-specific peaks for BCP at 960 cm⁻¹ and 1048 cm⁻¹ for CPP crystals.

2.6. Histological and Immune-Histological Stainings

Paraffin-embedded sections from human tendon tissue were deparaffinized and rehydrated using Xylene (Carl Roth, Karlsruhe, Germany) and a degrading ethanol series. Von Kossa staining was performed to visualize the localization and extent of calcification within the tendon tissue. Deparaffinized sections were stained with silver nitrate (5%, 1 h, Carl Roth, Karlsruhe, Germany), washed with distilled water (4 $\times$ 5 min) and incubated with pyrogallol (1%, 3 h, Carl Roth, Karlsruhe, Germany). After washing (4 $\times$ 5 min) with distilled water, sections were incubated with sodium-thiosulfate (5%, 3 min, Carl Roth, Karlsruhe, Germany), washed with tap water (5 min).

A combination of von Kossa and Sirius Red staining was used to visualize the collagen fiber and fiber orientation in tissue samples with calcific deposits. After von Kossa staining protocol, slides were washed with phosphate-buffered saline (5 min, Sigma-Aldrich, Darmstadt, Germany) and stained with picosirius red (0.13% direct red (Sigma Aldrich) in 1.2% picric acid (Waldeck GmbH, Münster, Germany) 1 h), washed with (0.01 N, 2 min, Carl Roth, Karlsruhe, Germany), followed by washing with distilled water. After staining, slides were dehydrated, cover-slipped using Canada balsam (Carl Roth, Karlsruhe, Germany) and pictures were taken at an Axiovert microscope (Zeiss, Oberkochen, Germany).

2.7. Statistical Analysis

Data analysis was performed using GraphPad Prism version 10 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Normality of the data was assessed using the Shapiro–Wilk test. Based on the distribution, non-parametric tests were applied for the respective analyses. Comparisons between more than two groups were performed using the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. Correlations were assessed using Spearman’s rank correlation coefficient. Statistical significance was determined at level of $p \leq 0.05$ (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

3. Results

3.1. CT Patients Show Numerically Lower ROM Values, Particularly for Retroversion

The parameters in Table 2 show numerical trends toward lower ROM values in CT patients compared with controls, particularly for retroversion. However, no significant differences were observed between the groups.

Table 2. ROM values in CT patients and controls. FE: forward elevation, RV: retroversion, ABD: abduction, ADD: adduction, IR: internal rotation, ER: external rotation. The numbers in the brackets indicate the total range of motion measured within the cohort. No significant differences were observed between the groups (two-way ANOVA $p = 0.7$).

Cohort	FE (°)	RV (°)	ABD (°)	ADD (°)	IR (°)	ER (°)
Calcific tendinitis (n = 33)	87.88 ± 25.00 (50–160)	17.73 ± 12.32 (0–40)	108.18 ± 41.19 (40–170)	23.94 ± 10.06 (5–40)	52.27 ± 23.49 (10–90)	33.03 ± 14.52 (10–70)
Control samples (n = 12)	97.50 ± 30.79 (60–160)	29.17 ± 21.51 (10–90)	117.50 ± 37.93 (40–170)	25.83 ± 7.93 (20–40)	51.67 ± 22.09 (20–90)	29.17 ± 15.64 (20–90)

3.2. Descriptive Overview of Comorbidities in CT Patients and Controls

As part of the initial medical history taken at patient admission, comorbidities were recorded (Table 3). Several comorbidities were observed more frequently in CT patients than in controls, particularly high blood pressure (51.5%) and disorders of fat metabolism (21.2%). Diabetes mellitus and hypertension were also numerically more common in CT patients. However, these observations should be interpreted descriptively due to the limited cohort size. In addition, CT patients (57 ± 9) were significantly older than control patients (40.5 ± 14) ($p < 0.0001$).

Table 3. Descriptive overview of comorbidities in the patient cohort. Due to multimorbidity, the total number of comorbidities exceeded the total number of patients.

	CT (n = 33)		Control (n = 12)	
	In absolute numbers	In %	In absolute numbers	In %
High blood pressure	17	51.25	4	33.33
Other cardiovascular diseases	4	12.12	0	0
Fat metabolism disorders	7	21.21	1	8.33
Diabetes	5	15.15	1	8.33
Inflammatory joint diseases	3	9.09	0	0
Others	14	42.42	7	58.33

3.3. The Histological Detected Calcified Area on Tendon Cross-Sections Showed a Correlation with the Volume of Calcific Deposits of MRI Scans

Two patients exhibited a clearly circumscribed and dense deposit that is characteristic of the formative phase (Gärtner type I). A clearly circumscribed deposit with an inhomogeneous structure or a vague bordered deposit with a homogeneous appearance was found in 14 patients (Gärtner type II). A cloudy and translucent deposit was observed in 17 patients classified as resorptive Gärtner type III. Representative X-ray images are shown in Figure 1A. In the next step, von Kossa stainings were performed (Figure 1B). In the tendon cross-sections harvested from control patients, small, calcified spots were shown in the histological analysis that did not appear on MRI scans. The quantification of the von Kossa-positive-stained area showed an increased calcific deposit area in Gärtner type II and type III compared to the control group. The mean calcification of the controls (2.3%) was significantly lower compared to the mean calcification of the CT group (28.0%). Interestingly, we did not observe differences in the amount of histological calcification between the Gärtner groups (Figure 1C). We observed a significant correlation between the radiological size of the calcified deposit and the amount of calcification detected in histology (Figure 1D). In the next step we analyzed the correlation between the

pain and the amount of calcification. We observed a significant increase in pain, measured by the VAS-Score, with an increase in histological calcification ($r = 0.33$, $p = 0.03$) (Figure 1E). The mean duration of shoulder pain until the time of preoperative assessment was 22.6 ± 3.3 months. However, when comparing the different Gärtner stages, we did not observe a correlation of pain with the different disease stages (Figure 1F).

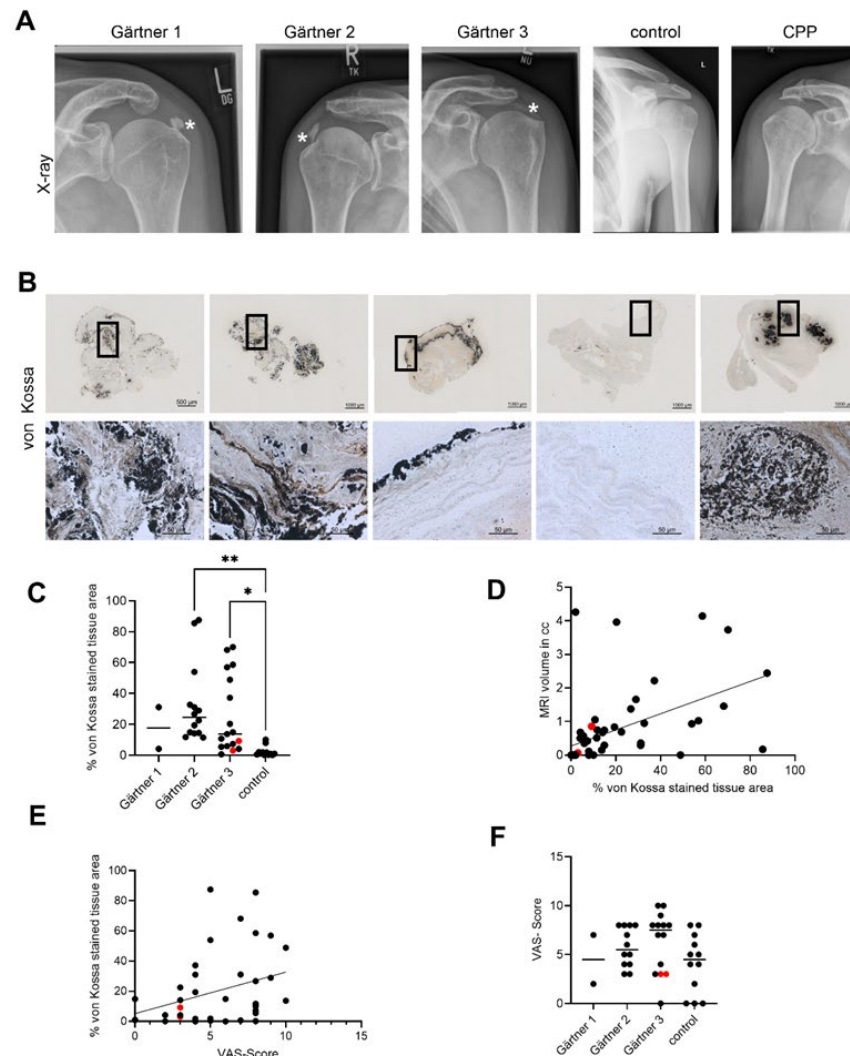


Figure 1. Comparison of the calcific deposit size in tendons categorized by the Gärtner classification. (A) Representative X-rays of CT patients divided according to the Gärtner classification. The asterisks mark the calcified deposit on the X-ray. (B) Representative von Kossa stainings of calcified deposits in patients with Gärtner type I, II, and III, control tissue without CT, and tendons with CPP-deposition. Scale bars: 500/1000 μm and 50 μm . (C) Quantification of the von Kossa-stained area (%) according to Gärtner type I, II, and III compared with the control group. (D) Positive correlation between the volume of calcium-containing deposits measured by MRI (cc) and von Kossa-stained area (%) ($n = 45$) (Spearman $r = 0.663$, $p < 0.001$). (E) Positive correlation between calcified tissue area and VAS pain score (Spearman $r = 0.385$, $p = 0.022$). (F) No significant influence of the Gärtner type on VAS pain score. CPP-positive tendons marked in red.

3.4. The Predominant Crystal Type in Tendons from CT Patients Was BCP

The high degree of collagen fiber orientation found in the control samples without calcific deposits (Figure 2A–E) was reduced in tendons from CT patients. Gärtner type I showed small calcific deposits and a loss of the collagen fibril structure and orientation (Figure 2A). Large calcific deposits and the loss of the typical collagen fibril structure were characteristic of tissue samples classified as Gärtner type II (Figure 2B). Tissue samples

classified as Gärtner type III showed collagen fibrils with the tendency of fiber alignment along the resorbing calcific deposits (Figure 2C). The tendon sample with CPP crystals (Figure 2D) showed local calcific deposits in areas with no collagen fibrils, while collagen fibers were maintained in areas without calcific deposits. Since tendon orientation during manual embedding and sectioning of small tissue samples cannot be fully standardized, differences in collagen fiber orientation were interpreted cautiously. To visualize the morphology of the calcific deposits, SEM images are shown. Arc and fragmented shapes of calcific crystals were observed in the formative phase (Gärtner type I).

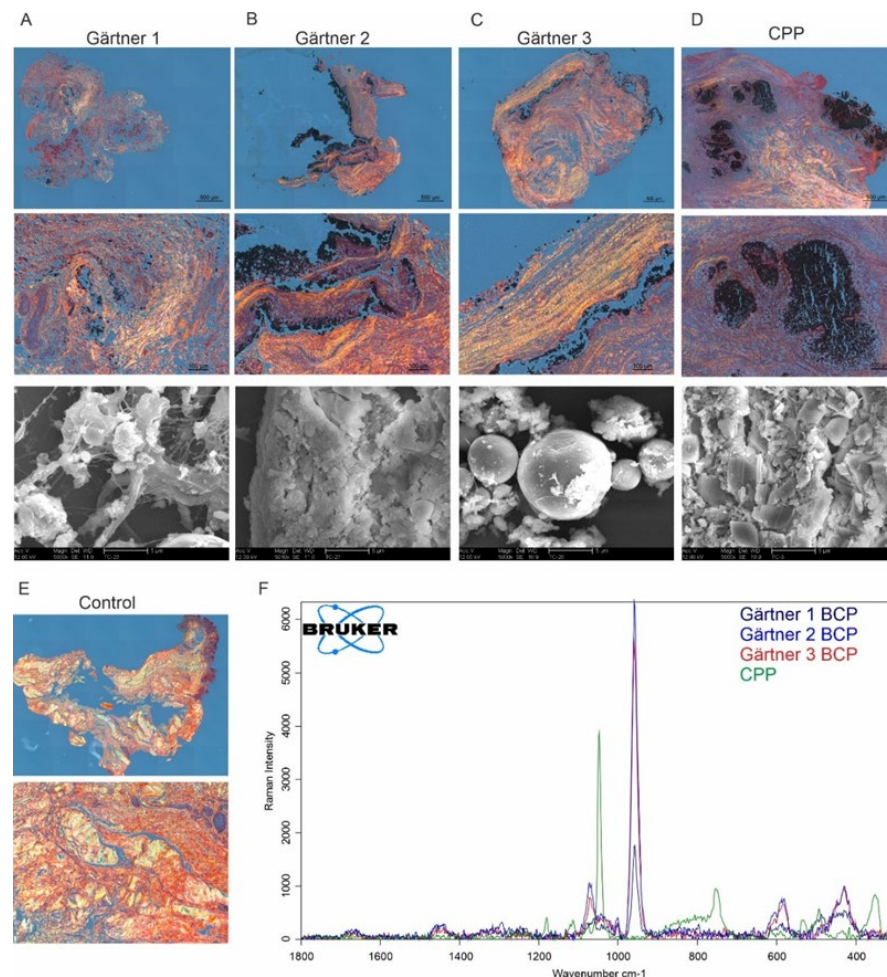


Figure 2. Identification of the crystal type in calcific deposits in the shoulder of CT patients using Raman spectroscopy and SEM imaging. Von Kossa Sirius red staining was used to visualize the location and collagen fibrous structure in tissue samples with calcific tendinitis. Representative images of tissue samples with (A) Gärtner type I, (B) type II, (C) type III, (D) CPP calcific deposits. And (E) control without calcified deposits. Scale bar 500/100 μm and SEM 5 μm . (F) Representative Raman spectra with the characteristic BCP specific peak at 960 cm^{-1} and CPP specific peak at 1048 cm^{-1} .

The calcified deposits in Gärtner type II are densely packed nodular crystals. The crystals in Gärtner type III samples were rounder and more fragmented compared to Gärtner type II. In one sample, we observed CPP-based calcification exhibiting a mixture of spike or rod-shaped crystals as well as nodular and densely packed structures (Figure 2D). The control sample is shown in Figure 2E.

To identify the calcium-containing crystal type in more detail, we applied confocal Raman spectroscopy (Figure 2F). We identified BCP deposition with its characteristic peak at 960 cm^{-1} in most patients (35/45). Two patients (2/45) exclusively contained CPP

deposits in the tendon sample indicated by the CPP-associated peak at 1048 cm^{-1} . In some patients (8/45), however, we could not detect any calcifications, which were all patients from the control cohort.

4. Discussion

Calcific tendinitis is a disease characterized by deposits in the tendons, leading to pain, inflammation, and limited mobility. Our CT cohort shows an increased level of multimorbidity compared with the control cohort, with high blood pressure and an increased percentage of metabolic disorders. This trend of comorbidities being more frequent in the CT cohort was confirmed in our study in relation to diabetes mellitus. Previous studies showed that diabetes mellitus patients exhibited a 27% increased risk of developing CT [19]. A large proportion of the affected tendons included in this study are located at the attachment of the M. supraspinatus. This observation is consistent with descriptions in the current literature [3]. Ideally, the amount of calcification measured in MRI is consistent with the amount of calcification measured in histological staining. Variations in the volume measured by MRI could occur due to the disjointed density and variance in thickness of tendon tissue in relation to the size of the depot. However, the MRI depot size and histological volume determination correlate significantly.

In past studies, the calcified depot size correlated with the pain symptoms [20]. In our cohort we also observed a correlation of histologically assessed calcification with pain. The basis for locating and quantifying the calcification histologically was a von Kossa staining. A large proportion of our patient cohort belonged to the Gärtner-type II and III group, characterized by poorly defined, inhomogeneous deposits. We did not observe the previously proposed dependence of pain intensity on different Gärtner stages [16]. This difference might be due to the comparably small cohort assessed in this study.

Calcific deposits identified within SEM analysis (within known ranges of $1\text{--}20\text{ }\mu\text{m}$ [21]) were further analyzed using Raman spectroscopy. BCP was the predominant crystal type of calcific deposits underlining the *in vivo* occurrence in CT; however, CPP was also detected. Grases et al. identified types of carbonated hydroxyapatite as well as inhibitors of calcification [22]. In rare cases, calcium pyrophosphate (CPP) crystals in CT have been described previously [13,23,24]. The absence of radiographic CPP detection despite histological and spectroscopic identification may be attributable to methodological differences in sensitivity and spatial resolution between the applied techniques. Raman spectroscopy and histological analysis enable microscopic detection of focal crystal deposits, whereas conventional radiography may not reliably detect deposits with low calcification burden or diffuse mineral distribution. Similar observations have been described for cartilage, in which smaller calcifications were not detected by X-ray but only by histological staining [25].

While BCP is the usual type of crystal being present in CT, CPP can present as acute or chronic tendinitis, sometimes at atypical sites such as the triceps, gluteus maximus, or biceps femoris tendons. CPP-related calcific tendinitis may also be associated with systemic diseases (e.g., SLE) [13] but also diabetes [19]. This was partially also observed in the two CPP patients in our cohort; one was diagnosed with lupus erythematosus. However, the second patient did not exhibit one of these comorbidities.

In previous studies, the presence of CPP was detected using CPP crystal detection in polarized light microscopy or dual-energy computed tomography [23]. This is, to our knowledge, the first study combining specific calcium-containing crystal identification using SEM-EDS and Raman spectroscopy with clinical and imaging parameters.

This study has some limitations that need to be kept in mind when interpreting the data. First, the patients in the control group were younger as in the CT group. An age-

related influence on the tendon homeostasis, therefore, could be possible. Second, the crystal identification was performed on histological sections, which represent only a very small part of the total tissue sample and might not be representative of the whole tendon. Therefore, we cannot exclude that other crystal types might also be present in other parts of the tissue.

5. Conclusions

Calcific tendinitis is mainly associated with BCP deposition, but rare cases of CPP can occur. The predominance of BCP crystals provides a basis for future mechanistic studies. In addition, calcification extent correlated with VAS pain score, suggesting that quantitative calcification assessment may serve as an imaging-based surrogate marker of disease burden. Metabolic comorbidities, including diabetes mellitus, were descriptively more frequent in CT patients than in controls.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request. Data are not publicly available due to privacy and ethical restrictions.

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Abbreviations

CT	Calcific tendinitis
SEM EDS	Scanning Electron Microscopy and Energy Dispersive X-ray Spectrometer
BCP	Basic calcium phosphate
CPP	Calcium pyrophosphate
BMI	Body mass index
SSP	Musculus supraspinatus
ISP	Musculus infraspinatus
SSC	Musculus subscapularis
VAS	visual analog scale
ROM	range of motion
FE	forward elevation
RV	retroversion
ABD	abduction
ADD	adduction

IR	internal rotation
ER	external rotation
fsPDWI	fat-suppressed proton density weighted images
T2WI	T2-weighted images
kV	kiloVolt
MRI	Magnetic resonance imaging
SLE	Systemic lupus erythematosus

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