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Research Article

A Comparative Microscopic Study of Apatite Rheumatism, Chondrocalcinosis and Synovial Chondromatosis - HA and CPPD Induced Metabolic Disorders

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Abstract

Introduction: Apatite rheumatism (AR), chondrocalcinosis (Ch-C) and primary synovial chondromatosis (prSynCh) are metabolic arthropathies caused by hydroxyapatite (HA) and/or by calcium pyrophosphate dihydrate (CPPD) crystal deposition.

The crystal deposits can be connoted with amorphous calcium phosphate [$Ca_3(PO_4)_2$], calcium carbonate [$CaCO_3$] deposits, chondroid and/or bone formation.

Objective of the Study: The object of this microscopic study was

(1) To ascertain the presence of HA and CPPD crystal deposits in AR, Ch-C and prSynCh viewed under polarized light in conventionally fixed tissue sections stained with HE, and with the technique of Bély and Apáthy (2013).

(2) To appraise the mineralization, chondroid and/or bone formation in tissue sections of AR, Ch-C and prSynCh with HE, Alizarin Red S, and the von Kossa reaction.

Patients and Methods: At the National Institute of Rheumatology and at the Hospital of the Order of the Brothers of Saint John of God surgical specimens of 101,855 patients were examined histologically between 1985 and 2010; among them were 5 patients with the clinical diagnosis of AR (0.0049%), 16 patients with Ch-C (0.016%), and 20 patients with prSynCh (0.020%).

Tissue samples of 47 joints (28 knee, 10 hip, 4 shoulder, 3 elbow, 2 wrist) of 41 patients with AR, Ch-C or with prSynCh were selected for this study.

Results: HA and/or CPPD crystals were found only occasionally in a few tissue sections stained with HE, while different amounts were demonstrated with Bély and Apáthy's non-staining technique (2013) in all patients with the clinical diagnosis of AR, Ch-C or prSynCh.

The amount of deposited calcium phosphate and/or carbonate was minimal in patients with prSynCh compared to the mineral deposits of patients with AR or with Ch-C.

Abundant chondroid and/or bone formation was characteristic to prSynCh in contrast with AR or with Ch-C.

Conclusion: The sensitivity of the non-stained section technique is a significantly better method for detection of HA or CPPD crystals than the HE stain.

The authors assume that prSynCh is an abnormal variant of an HA and CPPD induced metabolic disorder with reduced mineralization capabilities, in contrast to AR or Ch-C; the deficient mineralization is replaced by chondroid and/or bone formation.

Keywords: *Apatite Rheumatism; Chondrocalcinosis; Primary Synovial Chondromatosis; Conventional Stains and Histochemical Methods; Non-Staining Technique*

Abbreviations

AR: Apatite Rheumatism; Ch-C: Chondrocalcinosis; prSynCh - Primary Synovialis Chondromatosis; HA: Calcium Hydroxyapatite - [$Ca_5(PO_4)_3(OH)$]; CPPD: Calcium Pyrophosphate Dehydrate - [$Ca_2P_2O_7.2H_2O$]; MSU: Monosodium Urate Monohydrate [$NaC_5H_3N_4O_3{\cdot}H_2O$] Crystal - Crystalline Monosodium Salt of Uric Acid [$C_5H_4N_4O_3$]; HE: Hematoxylin Eosin; Ts: Tissue Samples; Pr. n0/y: Protocol Number/Year

Introduction

According to the pertinent literature apatite rheumatism (AR), chondrocalcinosis (Ch-C), and primary synovial chondromatosis (prSynCh) of unknown origin are distinct arthropathies.

Based on our previous studies the AR, Ch-C and prSynCh are of metabolic origin caused by deposition of hydroxyapatite [$Ca_5(PO_4)_3(OH)$] (HA) and/or by calcium pyrophosphate dihydrate [$Ca_2P_2O_7.2H_2O$] (CPPD) crystals [1-5].

The crystal deposits can be connoted with amorphous calcium phosphate [$Ca_3(PO_4)_2$], calcium carbonate [$CaCO_3$] deposits, chondroid and/or bone formation [1-5].

Objective of the Study

The authors wished to ascertain the presence of HA and CPPD crystal deposits in AR, Ch-C and prSynCh viewed under polarized light in conventionally fixed tissue sections stained with HE, and according to Bély and Apáthy (2013) [1-5], furthermore they estimated the mineralization, chondroid and/or bone formation in tissue sections of AR, Ch-C and prSynCh with HE, Alizarin Red S, and the von Kossa reaction.

Patients and Methods

We examined histologically 10 joints of 5 AR patients (6 knees, 3 shoulders, 1 hip), on 16 joints of 16 Ch-C patients (8 knees, 4 hips, 2 wrists, 1 shoulder, 1 elbow), and on 21 joints of 20 prSynCh patients (14 knees, 5 hips, 2 elbows).

Formalin fixed and traditionally proceeded tissue sections were stained with HE [6], Alizarin red S staining (specific for calcium) [7,8] or von Kossa's reaction (specific for phosphate and/or carbonate) [7,9] and were compared with unstained with unstained sections according to Bély and Apáthy (2013) [1-5,10-12] (See below).

Appendix: Bély and Apáthy's "non-staining" technique [1-5,10-12]:

1. Tissue blocks of surgically removed specimens are fixed in 8% neutral buffered formalin (at pH 7.6 for > 24 hours at 20°C room temperature).
2. Tissue blocks are dehydrated in ethyl alcohol, and are embedded in paraffin using acetone as well as xylene - 5 µm sections are cut.
3. Prolonged deparaffinization (3 - 5 days) in a thermostat at 56°C (daily changing xylene).
4. Chloroform - methanol I. (1:1) solution for 1 hour.
5. Chloroform - methanol II. (1:1) solution for 1 hour or overnight.
6. Dehydration in ethyl alcohol (two changes of 96% alcohol I-II. 30 - 30 minutes), and using terpene xylene, as well as xylene, mounting in Canada balsam, cover slip.

Demographics of the patients, and the amount of amorphous mineral deposits, chondroid, osteoid and/or bone formations were assessed by conventional stains and reactions [13-16] (using a semiquantitative core system*), and were compared with the student (Welch) T-probe [17].

Prevalence of deposited crystals in tissue sections stained by conventional HE and in non-stained ones was compared with Pearson's chi-squared (χ^2) test [17] in order to evaluate the sensitivity of the non-staining technique. The difference between two cohorts of samples was regarded "significant" at an alpha level of 0.05.

Standard and unstained sections were examined with the light microscope (Olympus BX51) and under polarized light, respectively.

Results

The mean age of patients with the clinical diagnosis of AR was high at the time of surgery compared to the mean age of the patients with the clinical diagnosis of Ch-C (74.80 year versus 63.67 year; $p < 0.0363$) or compared to the patients with the clinical diagnosis of prSynCh (74.80 year versus 50.20 year; $p < 0.0001$); these differences were significant especially between females (Table 1 and 2).

Table 1 summarizes the demographics of patient cohorts with AR (n = 5), Ch-C (n = 16) or prSynCh (n = 20).

Clinical diagnosis	Number of patients (n = 41)	Mean age in years at surgery ± SD	Range (In years)
Apatite rheumatism (n = 5)	**5 of 41**	**74.80 ± 6.91**	**66 - 82**
Female	4	77.00 ± 5.60	69 - 82
Male	1	66.00	66.00
Chondrocalcinosis (n = 16)	**16 of 41**	**63.67 ± 21.17**	**39 - 81**
Female	14	62.08 ± 21.93	39 - 81
Male	2	74.00 ± 1.41	73 - 74
Primary synovial chondromatosis - (n = 20)	**20 of 41**	**50.20 ± 12.51**	**30 - 76**
Female	13	53.15 ± 10.49	40 - 74
Male	7	44.71 ± 14.90	30 - 76

Table 1*: Sex, mean age with SD and range (in years) of 41 patients with clinically diagnosed AR, Ch-C or prSynCh.*
Remark to table 1: The mean age of patients with the clinical diagnosis of AR, Ch-C or prSynCh was based on all operated cases.

*Remarks

Semi-objective score system for assessment of the amount of amorphous mineral deposits, chondroid, osteoid and/or bone formations in conventional stained tissue samples:

"0" - No mineral deposits, chondroid and/or bone formation.

"1" - Minimal mineral deposits, chondroid and/or bone formation.

"2" - Moderate mineral deposits, chondroid and/or bone formation.

"3" - Abundant (massive) mineral deposits, chondroid and/or bone formation.

Table 2 summarizes the p" values of significance (at an alpha level of 0.05) comparing the mean age of patient cohorts with the clinical diagnosis of AR (n = 5), Ch-C (n = 16) and prSynCh (n = 20).

Number of patients with the clinical diagnosis of AR (n = 5), Ch-C (n = 16) or prSynCh (n = 20)	p < 0.05
Pts. with AR n = 5 versus Ch-C n = 16	**0.0363**
Female n = 4 of 5 versus n = 14 of 16	0.0099
Male* n = 1 of 5 versus n = 2 of 16	–
Pts. with AR n = 5 versus prSynCh n = 20	**0.0001**
Female n = 4 of 5 versus n = 13 of 20	0.0001
Male* n = 1 of 5 versus n = 7 of 20	–
Pts. with Ch-C n = 16 of 41 versus prSynCh n = 20	**0.0074**
Female n = 14 of 16 versus n = 13 of 20	0.0920
Male n = 2 of 16 versus n = 7 of 20	0.0218

Table 2: *Level of significance ("p" value <0.05) comparing the mean age of 41 patients with the clinical diagnosis of AR, Ch-C or prSynCh. Remark to table 2: The differences were significant in the mean age of patients between AR and Ch-C, furthermore between Ch-C and prSynCh. Differences were not calculated between male patients with the clinical diagnosis of AR and Ch-C, because of a zero divisor.*

The prevalence of HA and CPPD crystals was detected in 74 tissue sections of 28 patients; tissue samples of 13 patients with prSynCh were not available for analysis.

In tissue sections stained with HE, the HA crystals were found only in 3 (10.71%) of 28 patients (in 3 of 74 tissue sections, 4.054%), and CPPD in 12 (42.85%) of 28 patients (in 15 of 74 tissue section, 20.27%) with the clinical diagnosis of AR, Ch-C, or with prSynCh (Table 3).

Different amounts of HA and CPPD crystals were demonstrated with Bély and Apáthy's non-staining technique (2013) in all 28 patients (100%) with the clinical diagnosis of AR, Ch-C or prSynCh.

In 74 unstained tissue sections of 28 patients HA crystals were found in 49, (66.216% of 74), and CPPD crystals in 44 (59.459% of 74) cases.

The prevalence of HA or CPPD crystals was more frequent in unstained sections than in the HE stained ones; the differences were significant regarding the CPPD crystals ($c = 1$, $\chi^2 = 7.8612$, $p < 0.005051$).

Amorphous calcium phosphate and/or calcium carbonate deposits, furthermore chondroid and/or bone formation was appraised in 94 tissue sections of 38 patients in specimens stained with HE, Alizarin red S or von Kossa reaction; tissue samples or sections of 3 patients with prSynCh were not available (Table 3).

The amount of amorphous calcium phosphate [$Ca_3(PO_4)_2$] and/or calcium carbonate [$CaCO_3$] deposits was different in all patient groups; the average value of scores in tissue samples of patients with the clinical diagnosis of AR was (1.800/Pts), with Ch-C (2.600/Pts), and with prSynCh (0.556/Pts).

The difference of mineralization was not significant between AR and Ch-C patients (1.80/Pts versus 2.60/Pts, $p < 0.0549$).

The amount of deposited minerals of patients with prSynCh was minimal compared to the mineral deposits of patients with AR (0.556 versus 1.800, $p < 0.0081$) or with Ch-C (0.556 versus 2.600, $p < 0.000000013$); the differences were significant.

Abundant chondroid and/or bone formation was characteristic to prSynCh (4.389/Pts); the differences were significant in comparison with AR (4.389/Pts versus 0.100/Pts; $p < 0.000000$) or Ch-C (4.389/Pts versus 0.133/Pts; $p < 0.00276$).

There was no significant difference in chondroid formation (with or without osteoid or bone formation) between AR and Ch-C patients (0.100/Pts versus 0.133/Pts; $p < 0.425$) or chondroid formation and/or bone formation per tissue samples of AR and Ch-C patients ($p < 0.426$).

Figure 1-3 demonstrate the characteristic small prisms and clusters of HA crystals and morphology of individual CPPD crystals.

The HA crystals are small birefringent prisms or small hexagonal rods of different sizes; single crystals are submicroscopic [13] or 50 - 500 nm [14], in clusters (fascicles) 1 - 5 µm [14] or 1.9 - 15.6 µm [15].

The CPPD crystals have a trapezoid shape. Their expected size range is 0.42 - 17.9 µm [16] or even larger (submicroscopic to 40 µm) [15].

Original magnifications of all figures correspond to the 24x36 mm transparency slide; the correct height: width ratio is 2:3. The printed size may be different; therefore, the original magnifications are indicated.

In unstained sections of 5 patients, we found crystals with different shapes and sizes than the typical HA and CPPD crystals, arranged like a hair braid (vortex) or in a way reminiscent of a shingle roof.

Crystals with such different shapes, sizes and arrangement occurred in all three patient groups (in 1 patient with the clinical diagnosis of AR, in 2 with Ch-C, and in further 2 patients with prSynCh).

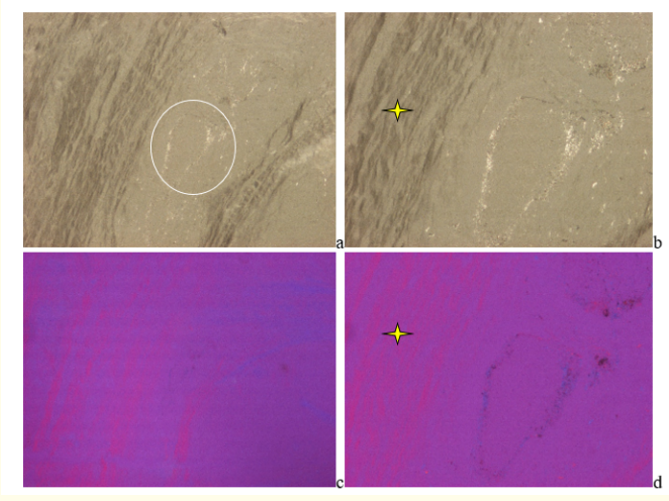

Figure 1a-d: *(3495/1997). Apatite rheumatism, knee joint, synovial membrane, small prisms of HA crystal deposits with weak birefringence (white ellipse). Under polarized light the direction of birefringence is positive, like that of collagen fibers (yellow star). (a) Unstained sections, viewed under polarized light, x100, (b) same as (a) x200, (c) Unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x100, (d) same as (c) x200.*

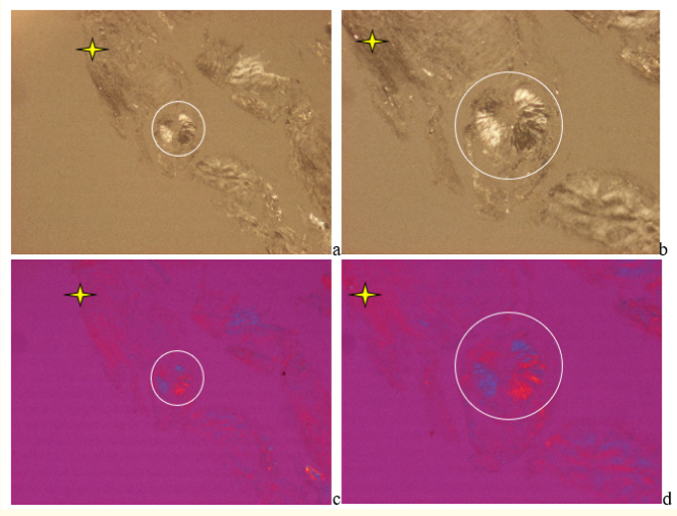

Figure 2a-2d: *(431/2016). Synovial osteo-chondromatosis, knee joint, advanced stage of HA crystal deposition. The rod-shaped prisms of HA crystals are arranged in larger spheroid microaggregates (white ellipse). Under polarized light the direction of birefringence is positive according to the long axis of HA crystals, like that of collagen fibers (yellow star). (a) HA crystals, unstained sections, viewed under polarized light, x100, (b) same as (a) x200, (c) HA crystals, unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x100, (d) same as (c) x200.*

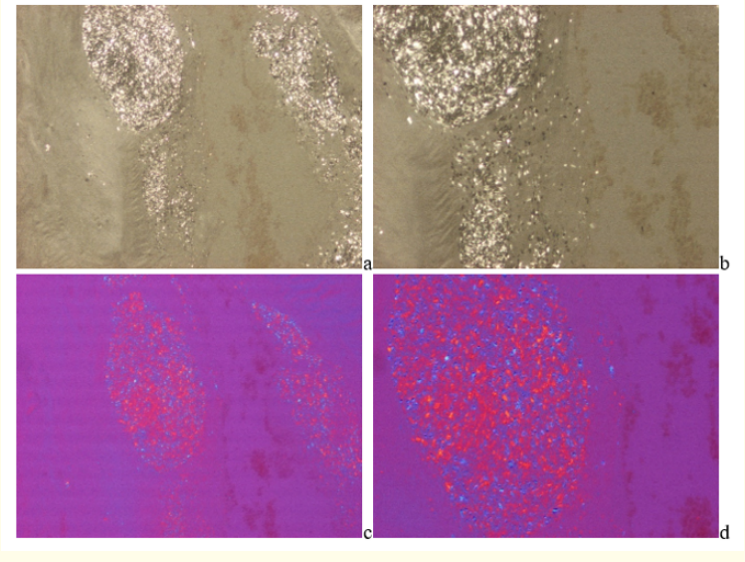

Figure 3a-3d: *(2739/1983). Chondrocalcinosis, hip joint, synovial membrane, with strong birefringent plane CPPD crystals. CPPD is typically a plane crystal with positive birefringence. Different forms may exist (hexagonal, trapezoid, rhomboid, parallelogram-shaped or fragments of these). (a) CPPD crystals, unstained sections, viewed under polarized light, x100, (b) same as (a) x200, (c) CPPD crystals, unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x100, (d) same as (c) x200.*

Their birefringence was stronger than that of HA crystals, and weaker than that of CPPD. The birefringence of the tight (closely) fitting parallel crystals was positive and negative in the same axis position (Table 3 and figure 4-8).

Figure 4-8 demonstrate the characteristics of these crystals of different shapes, sizes and arrangement other than that of HA or CPPD crystals.

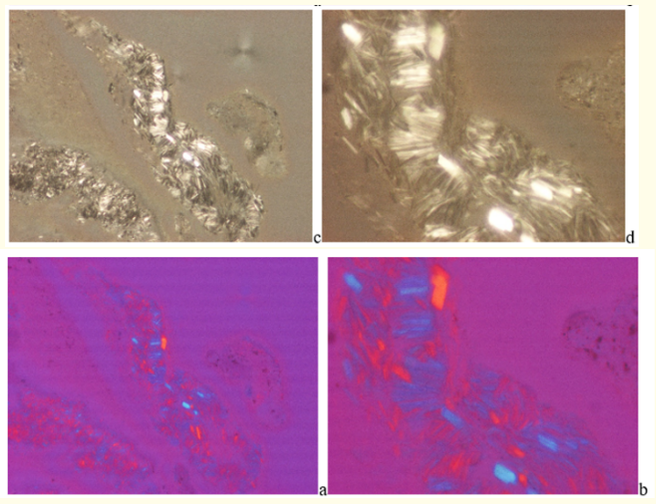

Figure 4a-4d: *(4443/1997). Apatite rheumatism, knee joint synovial membrane, CPPD and unidentified rod-shaped crystals. The birefringence of the sporadically scattered CPPD crystals is strong positive, compared to the tightly fitting unidentified crystals which in some cases are arranged like shingles of a roof. (a) unidentified crystals, unstained sections, viewed under polarized light, x200, (b) same as (a) x600, (c) unidentified crystals, unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x200, (d) same as (c) x600.*

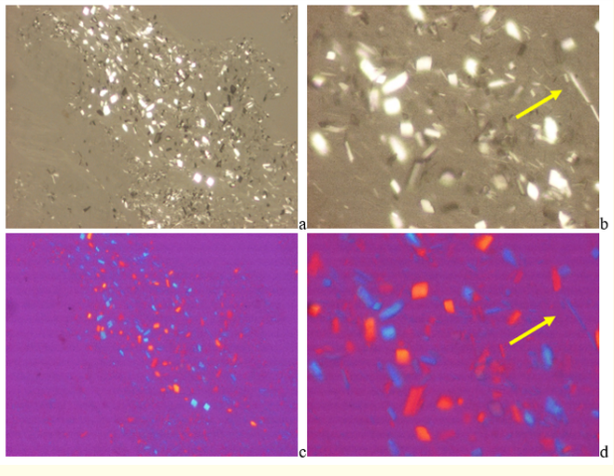

Figure 5a-5d: *(2417/2017). Apatite rheumatism, knee joint synovial membrane, typical rhomboid and parallelogram shaped CPPD crystals with positive birefringence, and unidentified long, narrow crystals, in the same direction of CPPD with positive birefringence; these crystals are different from MSU crystals, which are needle-shaped with a very strong negative birefringence. (a) unidentified long, narrow crystals (yellow arrow), unstained sections, viewed under polarized light, x200, (b) same as (a) x600, (c) unidentified long, narrow crystals (yellow arrow), unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x200, (d) same as (c) x600.*

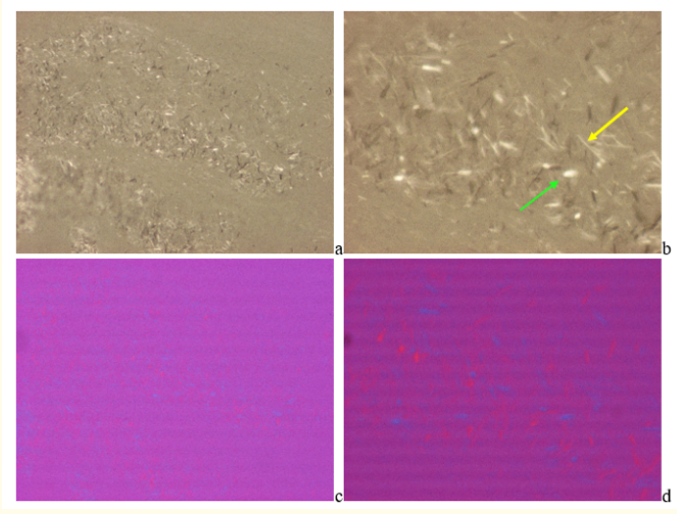

Figure 6a-6d*: (3097/1985). Apatite rheumatism, knee joint synovial membrane, relatively small parallelogram shaped CPPD crystals with a positive birefringence (green arow), and a large number of unidentified long, narrow crystals (yellow arrow). The birefringence of CPPD crystals is positive, and in the same direction the birefringence of these unidentified crystals is positive or negative. Their shape differs from that of MSU crystals, which are needle-shaped and have a very strong, consistently negative birefringence. (a) parallelogram shaped CPPD crystals with positive birefringence, and unidentified long, narrow crystals with positive or negative birefringence, unstained sections, viewed under polarized light, x200, (b) same as (a) x600, (c) unidentified long, narrow crystals, unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x200, (d) same as (c) x600.*

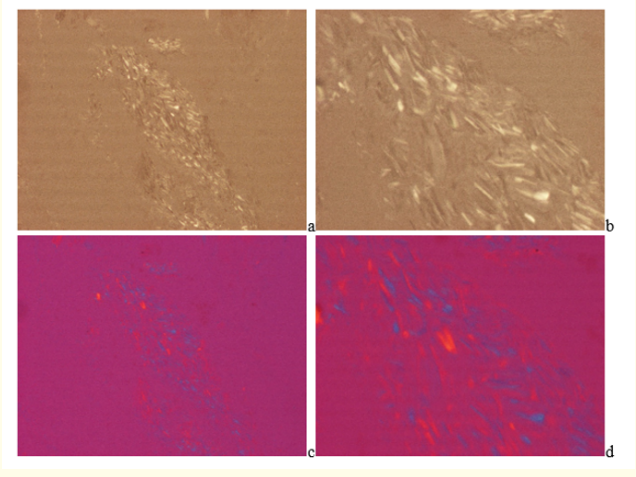

Figure 7a-7d: *(2569/2008). Synovial osteo-chondromatosis, knee joint synovial membrane, braid of unidentified rod-shaped crystals. The crystals are different from the typical CPPD crystals and are arranged in a braid; they are larger than CPPD crystals and their birefringence is positive or negative parallel to the long axis of the crystals. (a) unidentified crystals, unstained sections, viewed under polarized light, x200, (b) same as (a) x600, (c) unidentified crystals, unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x200, (d) same as (c) x600.*

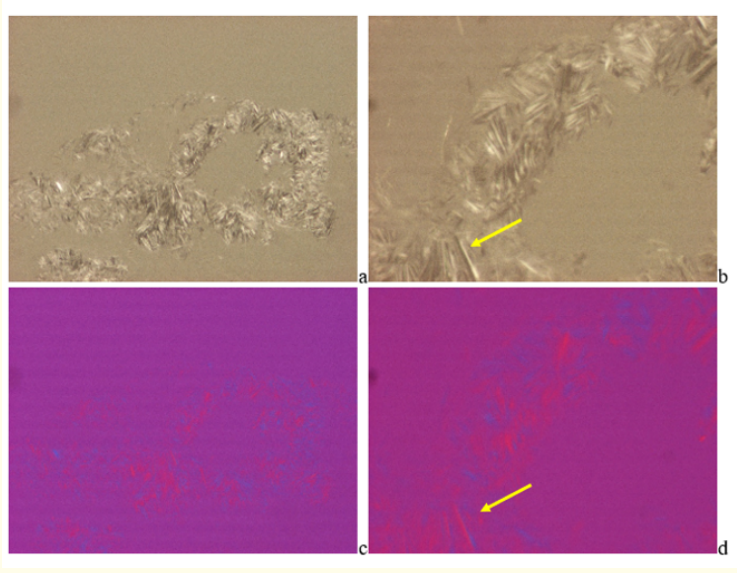

Figure 8a-8d: *(3097/1985). Chondrocalcinosis, knee joint synovial membrane, vortex of unidentified rod-shaped crystals. The crystals are longer than the typical CPPD crystals and are arranged like a vortex; their birefringence is positive or negative (yellow arrows) according to the long axis of the crystals. (a) unidentified crystals, unstained sections, viewed under polarized light, x200, (b) same as (a) x600, (c) unidentified crystals, unstained sections, viewed under polarized light, using Red I compensator, same fields as (a) x200, (d) same as (c) x600.*

Figure 9-11 demonstrate the characteristic ratio of mineral deposits and chondroid (with and without osteoid or bone) formation in AR, Ch-C and prSynCh.

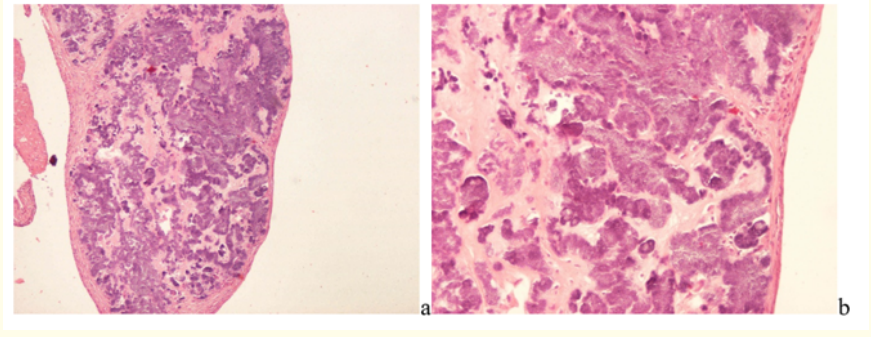

Figure 9a and 9b: *(4443/1997). Apatite rheumatism, knee joint synovial membrane, abundant calcification with minimal chondroid formation. (a) HE, viewed with the light microscope, x40, (b) same as (a) x100.*

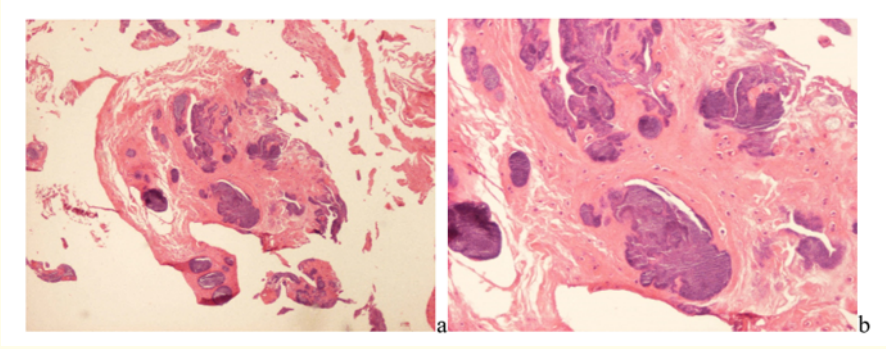

Figure 10a and 10b: *(192/1989). Chondrocalcinosis, knee joint synovial membrane, massive calcification with moderate chondroid formation. (a) HE, viewed with the light microscope, x40, (b) same as (a) x100.*

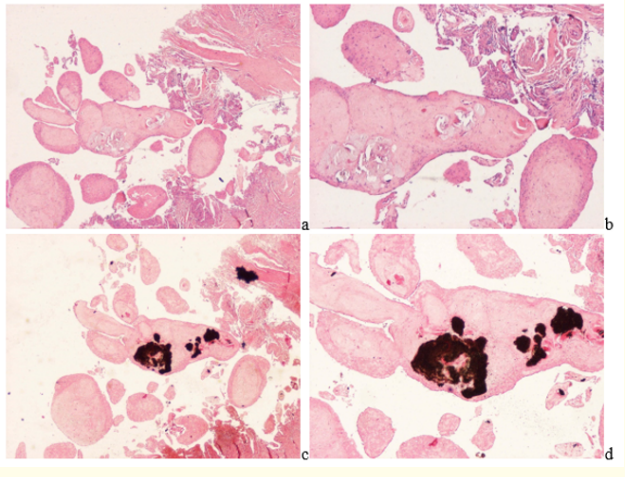

Figure 11a-11d: *(1727/2016). Synovial osteo-chondromatosis, knee joint synovial membrane with newly formed chondroid and osteoid nodules with moderate calcification between them. (a) HE, viewed with the light microscope, x20, (b) same as (a) x40, (c) von Kossa reaction, same as (a) x20, (d) same as (a) x40.*

Table 3 summarizes the ratio of HA and/or CPPD crystals in unstained sections, and the amount of amorphous calcium phosphate or carbonate deposits, furthermore it includes chondroid and/or osteoid formation in conventionally stained tissue sections of patients with the clinical diagnosis of AR, Ch-C and prSynCh.

Discussion

According to the literature HA and CPPD crystals are more common in the joints of older persons [18].

Patients with HA crystal induced arthritis are usually older than 70 years, and are more often women [19]. In a study of McCarty., *et al*. (1981) the mean age of four women with Milwaukee shoulder was between 63 and 90 years [20]. Dieppe., *et al*. (1984) reported apatite associated destructive arthritis in 11 women and in one man with a mean age of 66 to 83 years [21].

		Surgical specimens (Operated joints)					Ratio of HA and CPPD in various structures of joints Unstained tissue sections						HE stained tissue sections				
Number of patients with AR, Ch or prSynCh	Pr n0 /y	Knee	Hip	Shoulder	Elbow	Wrist	Synovial membrane	Punctatum	Capsule	Bursa	Tendon	Bone/Cartilage	Amorphous mineralization /Pts.	Amorphous mineralization /Ts.	Presence of HA or CPPD – HE/Pts	Chondroid – HE/Pts.	Osteoid and/or bone – HE/Pts.
AR	Pr no/y	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	Ca				
1	3495/97	1								90%			3	1	0	0	0
	3496/97	1								90%			3	1	0	0	0
2	4443/97	1					10%		90%				3	2	0	1	0
3	V/2170-19	1					90%		0%			0%	0	3	0	0	0
	V/2170-19			1			90%		0%			0%	0	3	0	0	0
	181-2019			1				90%					1	1	0	0	0
	181-2019			1				90%					1	1	0	0	0
4	C-17/2012	1						90%					1	1	0	0	0
5	2417/2014	1					60%		0%				3	2	0	0	0
	604/2015		1				60%		0%				3	2	0	0	0
		CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD					
1	3495/97	1								10%			3	1	1	0	0
	3496/97	1								10%			3	1	1	0	0
2	4443/97	1					Rod > 90%		0%				3	2	1	1	0
3	V/2170-19	1					10%		0%			0%	0	3	0	0	0
	V/2171-19			1			10%		0%			0%	0	3	0	0	0
	181-2019			1				10%					1	1	0	0	0
	181-2019			1				10%					1	1	0	0	0
4	C-17/2012	1						10%					1	1	0	0	0
5	2417/2014	1					40%		0%				3	2	0	0	0
	604/2015		1				40%		0%				3	2	0	0	0
Total n	HA+	6	1	3	0	0	5	3	1	2	0	0	18/10	18/17	0	1	0
of joints	CPPD+						5	3	0	2	0	0	1,80	1.059	3	1	0
Ch-C	Pr no/y	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA					
1	14/75					1					20%		3	1	0	0	0
2	2560/78			1			90%		90%			90%	1	3	0	0	0
3	631/1980		1				10%		0%			0%	ND	3	0	0	0
4	676/81				1		90%		60%	10%			3	3	0	0	0

5	834/83		1				90%		0%	0%	0%		3	4	0	0	0
6	2739/83		1				10%		10%			0%	3	3	0	0	0
7	2207/81	1					10%		90%				1	2	0	1	0
8	3097/85	1					10%		15%			5%	3	3	0	0	0
9	437/86		1				10%		5%			0%	3	3	1	1	0
10	3439/87	1					70%		0%				3	2	0	0	0
11	192/89					1	40%		40%				3	2	0	0	0
12	2086/94	1					10%		0%				3	2	0	0	0
13	3558/2002	1					10%		0%				3	2	0	0	0
14	169/2006	1					80%		0%				3	2	0	0	0
15	581/2007	1					60%		0%				3	2	0	0	0
16	477/2008	1					60%		0%	0%			1	3	0	0	0
		CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	CPPD					
1	14/75					1					80%		3	1	0	0	0
2	2560/78			1			10%		10%			1-1	1	3	0	0	0
3	631/1980		1				90%		5%			0%	ND	3	0	0	0
4	676/81				1		10%		1-1	10%			3	3	0	0	0
5	834/83		1				10%		0%	0%	0%		3	4	0	0	0
6	2739/83		1				90%		10%			0%	3	3	1	0	0
7	2207/81	1					Rod > 90%		1-1				1	2	0	1	0
8	3097/85	1					Rod > 90%		0%			1-1	3	3	0	0	0
9	437/86		1				90%		10%			0%	3	3	1	1	0
10	3439/87	1					30%		0%				3	2	0	0	0
11	192/89					1	60%		0%				3	2	0	0	0
12	2086/94	1					90%		10%				3	2	1	0	0
13	3558/2002	1					90%		0%				3	2	1	0	0
14	169/2006	1					20%		0%				3	2	0	0	0
15	581/2007	1					40%		0%				3	2	1	0	0
16	477/2008	1					40%		0%	0%			1	3	0	0	0
Total n	HA+	8	4	1	1	2	15	0	7	1	1	2	39/15	39/40	1	2	0
of joints	CPPD+						15	0	5	1	1	0	2,60	0.974	5	2	0
prSynCh	Pr no/y	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA	HA					
1	850/1982	1					ND		ND				0	2		3	0
2	2884/85	1					ND		ND				0	2		3	0
3	1283/87	1					ND		ND				1	2		3	2
4	141/88				1		ND		ND				1	2		3	2
5	1535/89	1					90%		0%				0	2	0	3	
6	1864/89				1		90%		0%				0	2	0	0	3
7	262/90	1															
8	833/90	1					ND		ND				1	2		3	2

9	3812/90	1					ND		ND				0	2		3	0
10	2724/93	1					ND		ND				0	2		3	0
11	5121/93		1				ND		ND				1	2		3	2
12	126/94	1					80%		0%				0	2	0	3	0
13	1083/95		1														
14	4789/95	1					ND		ND				0	2		3	0
15	5342/95		1				ND		ND				1	2		3	2
16	5154/97	1															
17	2569/2008	1					10%		90%				0	2	0	3	0
18	501/2015		1				90%		80%				1	2	1	3	0
19	1214/2015		1				80%		50%			0%	1	3	0	3	0
20	431/2016	1					20%		20%				2	2	0	3	2
21	1727/2016	1					80%		50%				1	2	1	3	2
		CPPD	CPPD	CPPD	CPPD	CPPD	CPPD	–	CPPD	–	–	CPPD					
1	850/1982	1					ND		ND				0	2		3	0
2	2884/85	1					ND		ND				0	2		3	0
3	1283/87	1					ND		ND				1	2		3	2
4	141/88				1		ND		ND				1	2		3	2
5	1535/89	1					10%		0%				0	2	0	3	
6	1864/89				1		10%		0%				0	2	0	0	3
7	262/90	1															
8	833/90	1					ND		ND				1	2		3	2
9	3812/90	1					ND		ND				0	2		3	0
10	2724/93	1					ND		ND				0	2		3	0
11	5121/93		1				ND		ND				1	2		3	2
12	126/94	1					20%		0%				0	2	0	3	0
13	1083/95		1														
14	4789/95	1					ND		ND				0	2		3	0
15	5342/95		1				ND		ND				1	2		3	2
16	5154/97	1															
17	2569/2008	1					Rod >90%		10%				0	2	0	3	0
18	501/2015		1				10%		20%				1	2	1	3	0
19	1214/2015		1				20%		50%			0%	1	3	1	3	0
20	431/2016	1					Rod >80%		0%				2	2	1	3	2
21	1727/2016	1					20%		50%				1	2	1	3	2
Total n	HA+	14	5	0	2	0	8	–	5	–	–	0	10/18	10/37	2	20	11
of joints	CPPD+						8	–	4	–	–	0	0.556	0.270	4		

Number of patients with AR, Ch or prSynCh	Pr n0 /y	Knee	Hip	Shoulder	Elbow	Wrist	Synovial membrane	Punctatum	Capsule	Bursa	Tendon	Bone / Cartilage	Amorphous mineralization /Pts.	Amorphous mineralization /Ts.	Presence of HA or CPPD – HE/Pts	Chondroid – HE/Pts.	Osteoid and/or bone – HE/Pts.
		Surgical specimens (Operated joints)					Ratio of HA and CPPD in various structures of joints Unstained tissue sections						HE stained tissue sections				

Table 3: *HA and CPPD crystals, amorphous calcium phosphate or carbonate deposits, as well as chondroid or osteoid formation in various tissue structures of different joints.*

Remark to table 3: Apatite rheumatism (AR), chondrocalcinosis (Ch-C), and primary synovial chondromatosis (prSynCh) diagnosed clinically.

Ten joints (17 Ts) of 5 patients with AR, 16 joints (40 Ts) of 16 patients with Ch-C, and 21 joints (37 Ts) of 20 patients with prSynCh were operated.

Surgical specimens of 18 joints with clinical diagnosis of prSynCh were available for histological analysis; tissue samples of 3 joints were not.

Seventeen tissue samples of 8 joints with clinically diagnosed prSynCh were available for unstained sections; tissue samples of 10 joints were not.

The amount of amorphous minerals was evaluated in 94 tissue samples of 38 patients with the clinical diagnosis of AR, Ch-C or prSynCh; tissue samples of 3 patients with prSynCh were not available.

Calcium phosphate [$Ca_3(PO_4)_2$] *and/or calcium carbonate [*$CaCO_3$*] deposits, furthermore chondroid and/or bone formation were appraised in tissue section stained with HE, Alizarin red S or the von Kossa reaction.*

A semiobjective score system was used to assess the amount of amorphous calcium phosphate and/or carbonate deposits, and for chondroid and/or bone formation ("0" - no mineral deposits, chondroid and/or bone formation, "1" - minimal, "2" - moderate, and "3" - abundant).

The average amount of mineral deposits was calculated for tissue samples in patients with AR, Ch-C or prSynCh. prSynCh.

The prevalence of HA and CPPD crystals was detected in 74 tissue sections of 28 patients; tissue samples of 13 patients with prSynCh were not available.

In tissue sections stained with HE the HA crystals were found only in 3 of 28 patients: in 1 with the clinical diagnosis of Ch-C (437/1986), and 2 with the clinical diagnosis of prSynCh (1214/2015, 431/2016).

CPPD crystals were present in 12 of 28 patients: in 3 with the clinical diagnosis of AR (3495/1997, 3496/1997, 4443/97), in 5 with Ch-C (2739/1983, 437/1986, 2086/94, 3558/2002, 581/ 2007), and in 4 with the clinical diagnosis of prSynCh (501/2015, 1214/2015, 431/2016, 1727/2016).

In unstained sections HA and CPPD crystals was demonstrated in all 28 patients (100 %) with the clinical diagnosis of AR, Ch-C or prSynCh; the HA crystals were present in 49, (66.216 %), and CPPD crystals in 44 (59.459 %) of 74 tissue samples.

Sporadic (scattered: 1-1) presence of CPPD crystals was assessed as negative.

AR: Apatite Rheumatism; Ch-C: Chondrocalcinosis; prSynCh - Primary Synovialis Chondromatosis; HA: Calcium Hydroxyapatite - [$Ca_5(PO_4)_3(OH)$*]; CPPD: Calcium Pyrophosphate Dehydrate - [*$Ca_2P_2O_7.2H_2O$*]; HE: Hematoxylin Eosin; Ts: Tissue Section; Pr. n0/y: Protocol Number/Year.*

Reginato and Yuvienco concluded that in CPPD induced diseases there is no major sex predominance; the prevalence of CPPD increases with age: it was 15 percent between 65 and 74 years, 36 percent between 75 and 84 years, and almost 50 percent in patients older than 84 years [22].

The mean age of patients with prSynCh is most often between 30 and 50 years, and men are affected twice as often as women [23].

The mean age of our patients with AR and in Ch-C corresponded to that of the literature, and females were also more often affected than males.

In contrast to the literature the range of mean age of our prSynCh patients was between 30 and 76 years, and women were nearly twice often affected than men (13 versus 7).

The high mean age of patients with clinically diagnosed AR at the time of surgery suggests that the small, more soluble, phagocytosed HA crystals are less symptomatic and their deposition is more tolerable for a longer period of time than that of the larger, less soluble, more irritative CPPD crystals.

The average age of the patients with clinically diagnosed Ch-C may be explained by the pronounced amorphous calcium phosphate [$Ca_3(PO_4)_2$] or calcium carbonate [$CaCO_3$] deposition, moderating the inflammatory reaction to crystal deposits.

The low mean age of patients with clinically diagnosed prSynCh at the time of surgery suggests that the massive chondroid or bone formation is not moderating the inflammatory reaction of crystal deposits, indeed, the newly formed calcified and/or ossified loose bodies themselves may cause early complaints (pain, limited motion etc).

In a progressive cumulative process crystal deposition starts in a tissue structure (synovial membrane, capsule, tendon sheet etc.) of one joint, and it later affects more structures of more joints with more pronounced crystal deposits.

According to the Wald equity [24], the "most common deposit is the earliest deposit" (the most common is the first).

Crystal deposition appears to begin with HA crystal deposition, followed later by CPPD deposition; the HA crystals were present in 49 of 74 tissue samples (66.22%), while CPPD in 44 of 74 (59.46%).

In our study the most frequent and massive crystal depositions occurred in all three patient groups in the synovial membranes of the knee, followed by other structures of other joints.

The knee was involved in all 28 patients; in 6 of 10 joints with AR, in 8 of 16 joints with Ch-C, and in 14 of 21 joints with prSynCh.

In the synovial membrane of the knees HA and CPPD deposits were found in all 28 patients; in 5 of 74 tissue samples with AR, in 15 with Ch-C, and in 8 with prSynCh.

According to our data crystal deposition begins in all three metabolic maladies in the synovial membrane of the knee, succeeded by other structures and other joints.

The deposited HA and CPPD crystals can provoke an intensive inflammatory reaction with clinical symptoms: sudden onset of severe pain, swelling, tenderness, and restricted motion of joints, with overlying redness and warmth; rapid progression may occur with joint destruction and instability [19,21,25-27].

The HA and CPPD crystal deposits in patients with AR or Ch-C are accompanied by pronounced amorphous mineral deposits, whereas in patients with prSynCh are not.

Chondroid and/or osteoid formation is exceptional and minimal in patients with AR or Ch-C, but it is the dominant basic characteristic of patients with prSynCh.

According to our interpretation, amorphous calcium deposition is a first-line, and chondroid and/or osteoid formation is a second-line fundamental mechanism in terms of crystal isolation and inflammation.

In our view the prSynCh is a defective variant of metabolic disorders, characterized by diminished calcium phosphate and/or carbonate presence.

Conclusion

Our study indicates that AR, Ch-C and prSynCh are crystal induced maladies, and belong to the same group of metabolic diseases.

The authors assume that the prSynCh is a defective variant of HA and CPPD induced metabolic disorder with reduced mineralization capabilities, in contrast to AR or Ch-C; the deficient mineralization is replaced by chondroid and/or bone formation.

The efficiency of non-stained histologic sections is a significantly better method for detection of HA or CPPD crystals than in HE stained ones.

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Volume 12 Issue 6 June 2023