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Research Article

Crystal Deposits in Apatite Rheumatism and Chondrocalcinosis – Microscopic Identification of Hydroxyapatite and Calcium Pyrophosphate Dihydrate Crystals with Standard Stains and Histochemical Reactions and with the Nonstaining Technique of Bély and Apáthy

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Abstract

Arthropathy induced by hydroxyapatite (**HA**) crystals (apatite rheumatism) and arthropathy induced by calcium pyrophosphate dihydrate (**CPPD**) crystals (chondrocalcinosis) are regarded as different metabolic diseases, and are considered distinct clinical entities.

In conventional **HE**-stained histological sections the more soluble, submicroscopic weak birefringent **HA** crystals are virtually non-existent, while the less soluble, large strong birefringent **CPPD** crystals occasionally may remain, supporting the clinicasl distinction between these two diseases.

The introduction of "non-staining" techniques by Bély and Apáthy (2013) into surgical pathology opened a new era in crystal diagnostics, and identification of crystals in different metabolic disorders.

The **aim** of this study was to describe the characteristic histology of **HA** and **CPPD** crystal deposits in tissue samples of patients with clinically diagnosed apatite rheumatism and chondrocalcinosis.

Patients and Methods: Conventional fixed and stained tissue sections were compared with unstained sections of **5** patients with clinically diagnosed **apatite rheumatism**, and **16** patients with clinically diagnosed **chondrocalcinosis**.

Results: In both disease crystals accumulated in the synovial membranes, in articular hyaline and/or fibrocartilage, joint capsules, bursae, tendon sheaths and in periarticular connective tissue with or without calcification.

Using Bély and Apáthy's non-staining technique (2013) different amount of **CPPD** and **HA** crystals were demonstrated in all patients with the clinical diagnosis of apatite rheumatism or chondrocalcinosis

Clinically diagnosed apatite rheumatism was characterized by the dominance of **HA** crystals, and the clinically diagnosed chondrocalcinosis by the dominance of **CPPD** crystals, with or without inflammatory reaction and phagocytosis. The **HA** and **CPPD** crystals were accompanied with more or less aboundant amorphous calcium phosphate or carbonate deposition, without remarkable inflammatory reactions.

Discussion: According to our results the **HA** crystal deposits were always accompanied with scattered or more or less amount of **CPPD** crystals, and **CPPD** crystal deposits were not detected without **HA** crystals. Our result support, that apatite rheumatism or chondrocalcinosis are progressive crystal induced diseases.

It seems to be, that "apatite rheumatism" or "chondrocalcinosis" starts with **HA** crystal deposition, followed later by **CPPD** deposition, with or without an intensive inflammatory reaction. The **HA** and **CPPD** crystal deposits may be accompanied with more or less amount of amorphous calcium phosphate or carbonate, without remarkable inflammatory reaction.

Conclusions: Apatite rheumatism and chondrocalcinosis are regarded clinically as different crystal induced metabolic diseases.

Using Bély and Apáthy's non-staining technique (2013) in both disorders various amount of **HA** and **CPPD** crystals were detected, accompanied by more or less amorphous calcium phosphate and carbonate deposits.

Our study indicates that apatite rheumatism and chondrocalcinosis are crystal induced diseases caused by the same basic metabolic disorder, moreover represent different stages of the same metabolic disorder.

The diagnosis of these metabolic diseases should be based on the quality of the dominant crystals in the deposits and not according to the overlapping clinical symptoms or localization.

Distinction between the two diseases seems to be of theoretical significance given present therapeutic possibilities.

Keywords*: Apatite Rheumatism; Chondrocalcinosis; Hydroxyapatite and Calcium Pyrophosphate Dihydrate Crystals*

Abbreviations

HA: Calcium hydroxyapatite – **[**$Ca_5(PO_4)_3(OH)$**]; CPPD**: Calcium pyrophosphate dehydrate **-** **[**$Ca_2P_2O_7.2H_2O$];

HE: **H**ematoxylin **E**osin; **CT**: **C**omputed **T**omography scan; **MRI**: **M**agnetic **R**esonance **I**maging; **ND**: **N**o **D**ata;

Pr n^0/y: Protocol number/year

Introduction

Arthropathy induced by hydroxyapatite [$Ca_5(PO_4)_3(OH)$] (**HA**) crystals (apatite rheumatism, hydroxyapatite rheumatism, hydroxyapatite arthritis, calcifying tenosynovitis, Milwaukee syndrome, calcific tendinitis, calcific bursitis, calcific periarthritis, periarthritis calcarea) and arthropathy induced by calcium pyrophosphate dihydrate [$Ca_2P_2O_7.2H_2O$] (**CPPD**) crystals (chondrocalcinosis, pseudogout, pyrophosphate arthropathy) are regarded as different metabolic diseases, and are considered distinct clinical entities, based on different clinical manifestations and symptoms [1-8].

The solubility of **HA** and **CPPD** crystals is different in conventional fixatives (aqueous formaldehyde solution), in alcohol, acetone, xylene and in aqueous solutions of dyes.

In conventional **HE**-stained histological sections the more soluble, submicroscopic (50-500 nm or in clusters 1-15 μm) weak birefringent **HA** crystals are virtually non-existent [9], while the less soluble, large (5 to 40 μm), strong birefringent **CPPD** crystals occasionally may remain [5-7].

In tissue sections stained with **HE**, the absence of **HA** crystals in apatite rheumatism and the presence of **CPPD** in chondrocalcinosis also supports the distinction between these two diseases.

Apatite rheumatisms and chondrocalcinosis may be accompanied by more or less abundant amorphous calcium phosphate [$CaPO_4$] or calcium carbonate [$CaCO_3$] deposition, which may mask the only exceptionally preserved **HA** or occasionally retained **CPPD** crystals.

The amorphous calcium phosphate or carbonate deposits (with or without **HA** or **CPPD** crystal contents) are characterized by nearly complete absence of inflammatory reaction.

The introduction of "non-staining" techniques by Bély and Apáthy (2013) into surgical pathology opened a new era in crystal diagnostics, and identification of crystals in different metabolic disorders [10].

This new, very sensitive method is suitable to identify cholesterol **[**$C_{27}H_{46}O$] (**CC**), hydroxyapatite [$Ca_5(PO_4)_3(OH)$] (**HA**), calcium pyrophosphate dihydrate [$Ca_2P_2O_7.2H_2O$] (**CPPD**), monosodium salt of uric acid [$C_5H_4N_4O_3$] (**MSU**) crystals, etc. in formalin fixed, paraffin embedded, unstained tissue sections viewed with polarized light [10-16].

The aim of this study was

1. To describe the characteristic histology of apatite rheumatism and chondrocalcinosis with conventional stains and reactions.

2. To identify **HA** [$Ca_5(PO_4)_3(OH)$] and **CPPD** [$Ca_2P_2O_7.2H_2O$] crystal deposits by non-staining techniques according to Bély and Apáthy in patients with clinical diagnosis of apatite rheumatism and chondrocalcinosis.

Patients and Methods

Surgical specimens (n = 13) and synovial needle biopsies (n = 2) of **5** patients with clinically diagnosed **apatite rheumatism** (average age in years 74.80, range 66 - 82, females 4, average age: 77.0, range: 69 - 82; male 1, average age 66.0), and surgical specimens (n = 35) of **16** patients with clinically diagnosed **chondrocalcinosis** (average age in years at biopsy 63.67, range 39 - 81, females 14, average age: 62.08, range: 39 - 81; male 2, average age: 74.0, range: 73 - 75) were studied.

Apatite rheumatism and chondrocalcinosis were clinically diagnosed.

The tissue blocks were fixed in an 8% aqueous solution of formaldehyde [$CH_2(OH)_2$] at pH 7.6 for > 24 hours at room temperature (20 C°) and embedded in paraffin. Serial tissue sections (5 microns) of apatite rheumatism and chondrocalcinosis were stained with **HE** [17], **Alizarin red S** (staining specific for calcium) [18, 19], and **von Kossa**'s reaction (specific for phosphate [$CaPO_4$] and carbonate [$CaCO_3$] [18, 20] and were compared with unstained ones [10].

Using **Bély** and **Apáthy**'s "non-staining" technique [10 – 15], the fixation of tissue blocks and embedding in paraffin were the same as with the standard staining's and reactions. Unstained tissue sections were deparaffinized, mounted and cover slipped with Canada balsam (descriptiom of method see Appendix below).

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

Appendix

Bély and Apáthy's "non-staining" technique [10 - 15].

1. Tissue blocks of surgically removed specimens were fixed in 8% neutral buffered formalin (at pH 7.6 for >24 hours at 20 Co room temperature).
2. Tissue blocks were dehydrated in alcohol, and were embedded in paraffin using acetone as well as xylene – 5 μm sections were 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 ethylalcohol (two changes of 96% alcohol I-II. 30-30 min.), and using terpene xylene, as well as xylene, mount in Canada balsam, cover slip.

Results

I. Microscopic characteristics of clinically diagnosed apatite rheumatism

(Hydroxyapatite [$Ca_5(PO_4)_3(OH)$] (**HA**) crystals induced arthropathy)

Apatite rheumatism (Hydroxy apatite arthropathy) was characterized by amorphous calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits of irregular shape in sections stained with HE, Alizarin red S or von Kossa's reaction, without demonstrable **HA** crystals, and with occasionally preserved CPPD crystals.

The calcium phosphate and/or calcium carbonate deposits in periarticular connective tissues, tendons or in synovial membranes were usually characterized by nearly complete absence of inflammation.

Free, independent **HA** crystals (with or without **CPPD** crystals, and/or amorphous calcium phosphate or carbonate mineral deposits) have been shown to cause inflammation.

Prepatellar mineral deposits of a 63-year-old female patient (Pr n^0/y: 3495-3496/1997) with the clinical diagnosis of apatite rheumatism are demonstrated by plain film radiography, CT and MRI in figure 1 to 3.

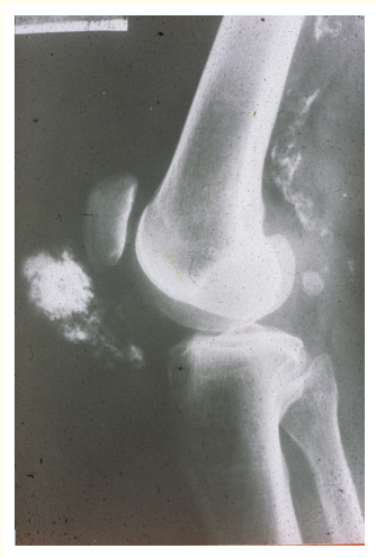

Figure 1: *Prepatellar bursitis with clinical diagnosis of apatite rheumatism, plain radiograph. Amorphous calcified prepatellar conglomerates and spotted (smudgy) calcification in the walls of popliteal and femoral arteries.*

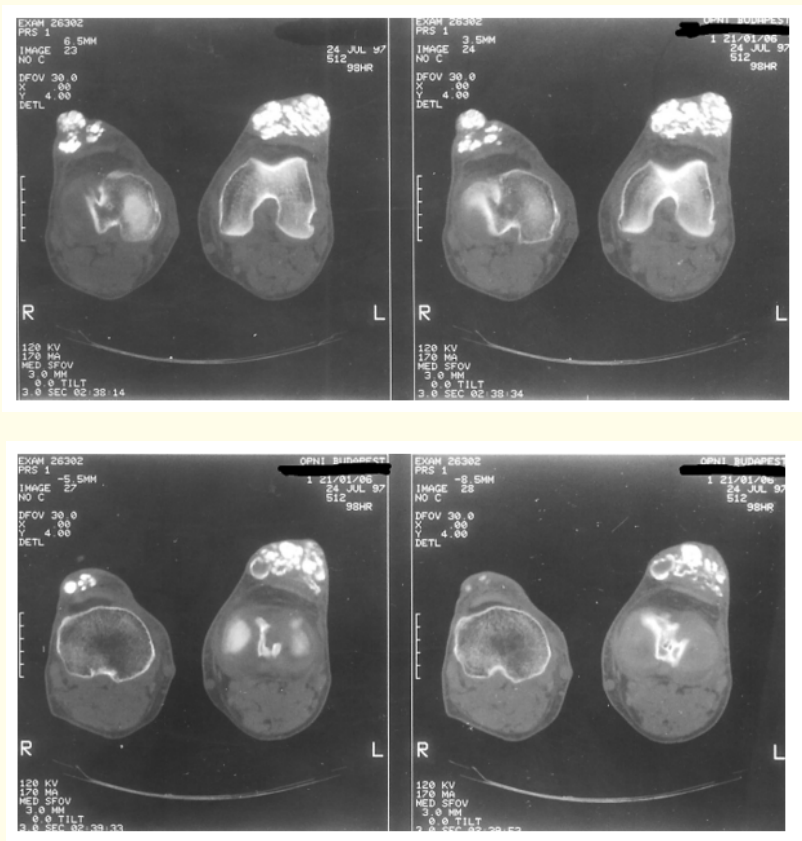

Figure 2: *Prepatellar bursitis of left and right knees, with the clinical diagnosis of apatite rheumatism. Amorphous prepatellar calcification on right side 3x3.5 cm, and on left side 4x7 cm.*

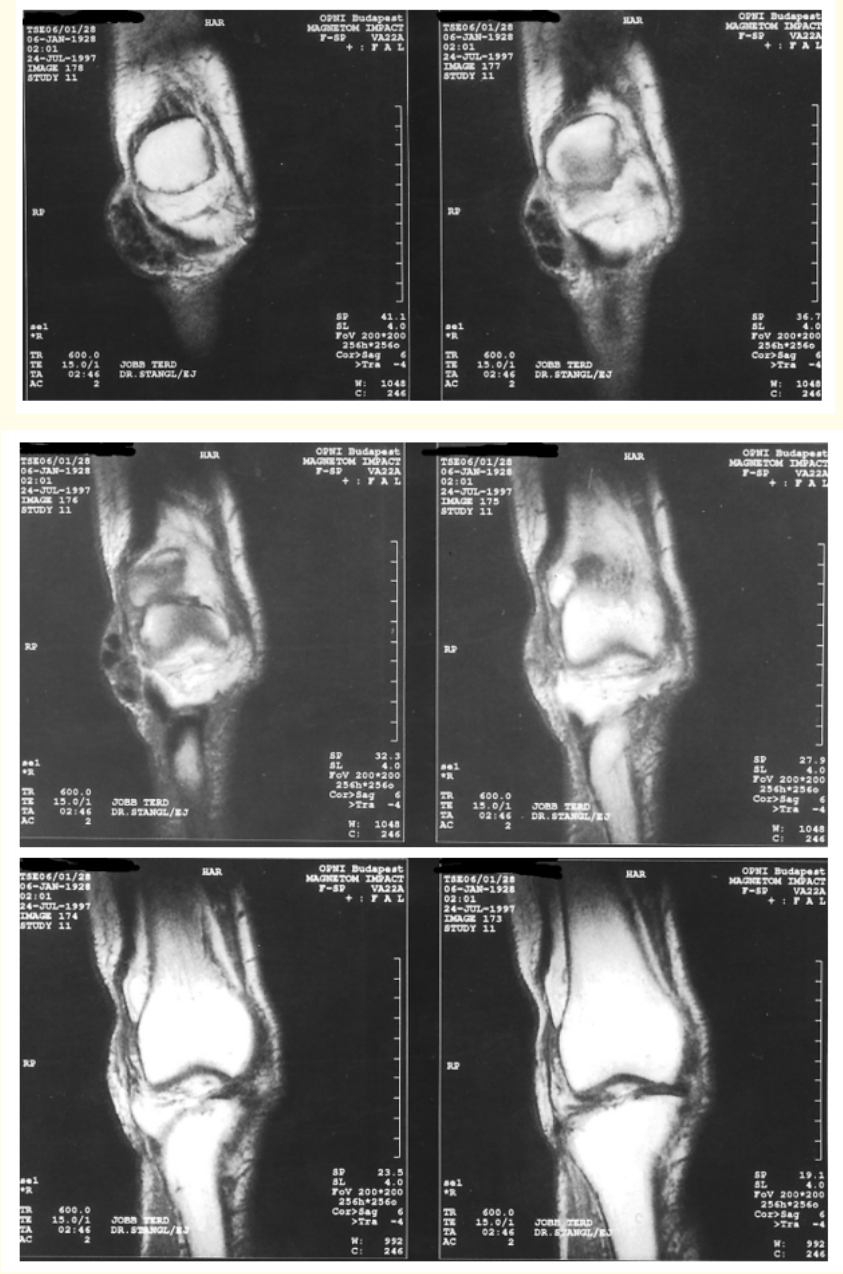

Figure 3: *Athritis involving both knees, with clinical diagnosis of apatite rheumatism, magnetic resonance image. Partial rupture of medial meniscus, bilateral chondromalacia, amorphous mineral deposits in bilateral prepatellar busae.*

Figure 4 horizontal histological sections of prepatellar bursa, surgical specimen (Pr n^0/y: 3495-3496/1997).

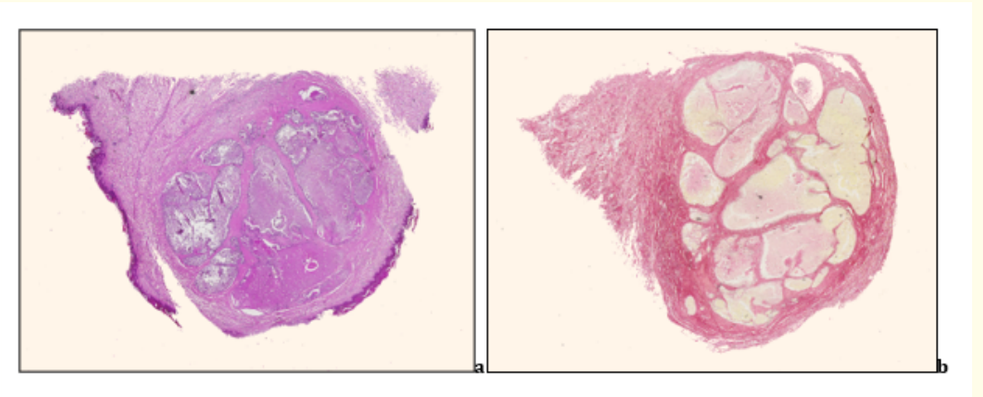

Figure 4: *Prepatellar bursitis, surgical specimen, horizontal section of prepatellar tissues, with clinical diagnosis of apatite rheumatism. Calcium phosphate and/or calcium carbonate deposits in prepatellar connective tissues, characterized by nearly complete absence of inflammation.*

(a) Mineral deposits in prepatellar bura, viewed with the light microscope, HE x1,

(b) Mineral deposits are surrounded by compact collagen fibres without inflammatory reaction, same as (a) Sirius red F3BA staining x1.

Figures 5 and 6 show the characteristic amorphous calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits in a patient (Pr n^0/y: 3495-3496/1997) with clinical diagnosis of apatite rheumatism.

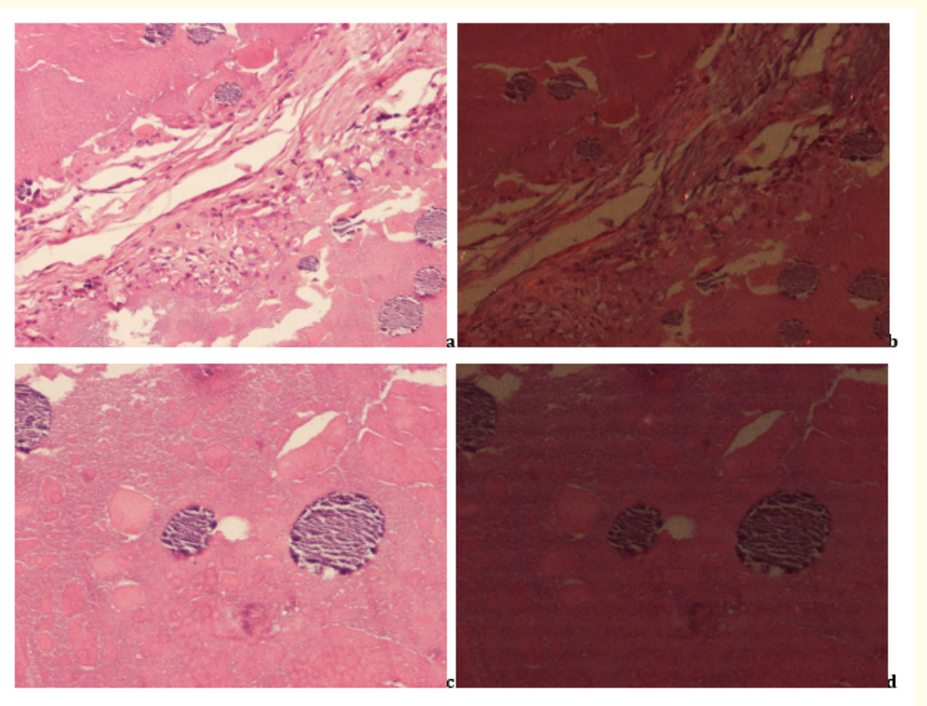

Figure 5a-d: *Prepatellar bursa, hydroxyapatite arthropathy, HA crystals, viewed with the light microscope and under polarized light, respectively.*

The HA crystals are accompanied by amorphous calcium phosphate, or calcium carbonate deposits of blue-violett colour.

The HA crystals are readily soluble and are not detected in traditionally fixed tissue specimens stained with HE; they are demonstrable in unstained sections of traditionally fixed tissue specimens (see bellow Figure 6a-d).

(a) HE, viewed with the light microscope x100, (b) HE, viewed under polarized light, same as (a) x100, (c) same as (a) x200 (d) same as (b) x200.

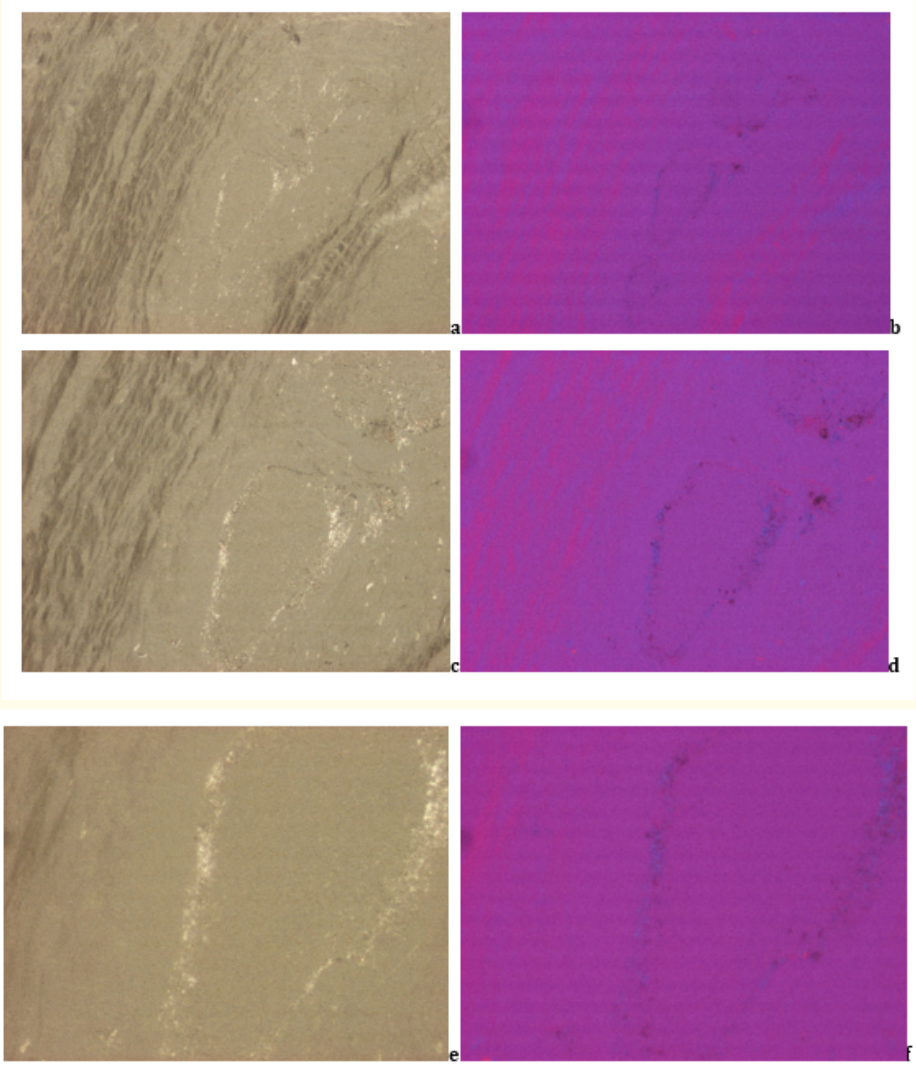

Figure 6a-f: *Prepatellar bursa, hydroxyapatite arthropathy, HA crystal fragments, unstained section, viewed under polarized light without Red I compensator: (a, c and e), and with Red I compensator: (b, d and f).*

The HA crystals are colorless, translucent fragments or small (50-500 nm), rod-shaped prisms. Under polarized light the intensity of birefringence of HA crystals is weak and positive.

Sporadically CPPD crystals may be also present, with a similarly positive but stronger birefringence (see Figure 8a-f).

Unstained sections, viewed under polarized light: (a) x100, (c) x200, (e) x400

Unstained sections, viewed under polarized light, using Red I compensator (same fields): (b) x100, (d) x200, (f) x400.

In tissue sections stained by HE, Alizarin red S or von Kossa's reaction the **HA** crystal aggregates are only exceptionally detected; **CPPD** crystals may be occasionally preserved.

In **unstained** sections viewed under polarized light both **HA** and **CPPD** crystals were detected in all patients with the clinical diagnosis of apatite rheumatism.

Apatite rheumatism was charactezized by large amounts of **HA** crystals (Figure 6a-f), accompanied by scattered **CPPD** cyrstals, except one patient in whom **HA** (60%) and **CPPD** crystals (40%) were present nearly in the same amount (Pr n^0/y: 604/2015).

In **unstained** sections viewed under polarized light the **HA** crystals were small (50 - 500 nm), rod-shaped (hexagonal with dipyramidal or rounded endings), and were arranged typically in 1 - 5 µm spheroid microaggregates or parallel groups (fascicles).

The birefringence of **HA** crystals was much weaker than that of **CPPD** crystals (Figure 8a-f).

With Red I compensator the **HA** crystals (like the **CPPD** crystals) showed a positive birefringence, like collagen fibers (Figure 6a-f).

The **HA** crystals, with or without amorphous amorphous calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits may provoke intensive inflammatory reaction with microphages (polymorphonuclear leucocytes) and macrophages (giant cells) (Figure 7a-d) (Pr n^0/y: 676/1981).

Figure 8a-f demonstrates the coexistent **HA** [$Ca_5(PO_4)_3(OH)$] and **CPPD** [$Ca_2P_2O_7.2H_2O$] deposit in synovial membrane (Pr n^0/y: 4443/1997).

Figure 9a-f demonstrates massive calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits of irregular shape in synovial membrane, stained by Alizarin red S, viewed by the light microscope, and under polarized light, respectively (Pr n^0/y: 4443/1997).

Figure 10a-d demonstrates massive calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits of irregular shape, sections of synovial membrane stained with von Kossa's reaction, viewed under the light microscope (Pr n^0/y: 4443/1997).

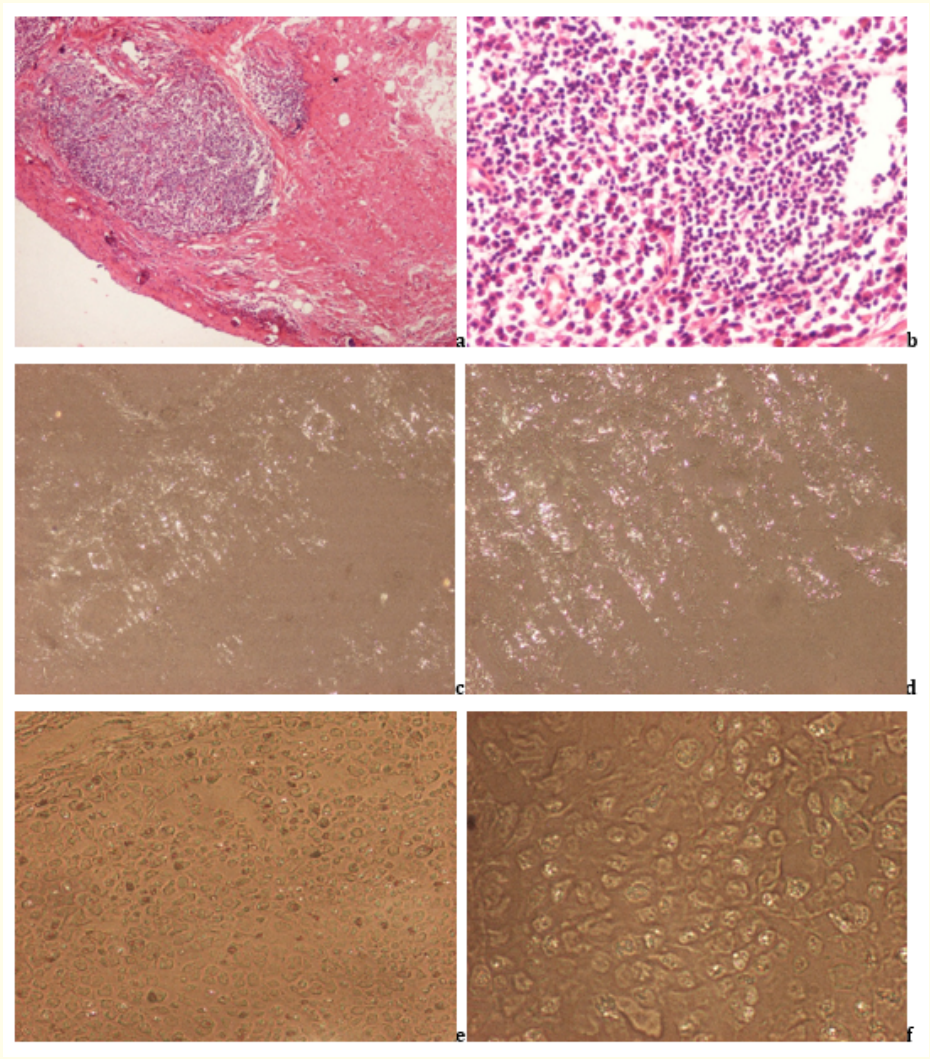

Figure 7a-f: *Prepatellar bursa, hydroxyapatite arthropathy, HA crystals, viewed with the light microscope. The HA crystals (with or without CPPD crystals are accompanied by an intensive acute-subacute inflammatory reaction, including macrophages with phagocytosed crystals.*

(a) HE, viewed with the light microscope x40, (b) same as (a) x200,

(c) same as (a), unstained sections, viewed under polarized light, x40, (d) same as (c) x100, (e) same as (d) x200, (f) same as (e) x600.

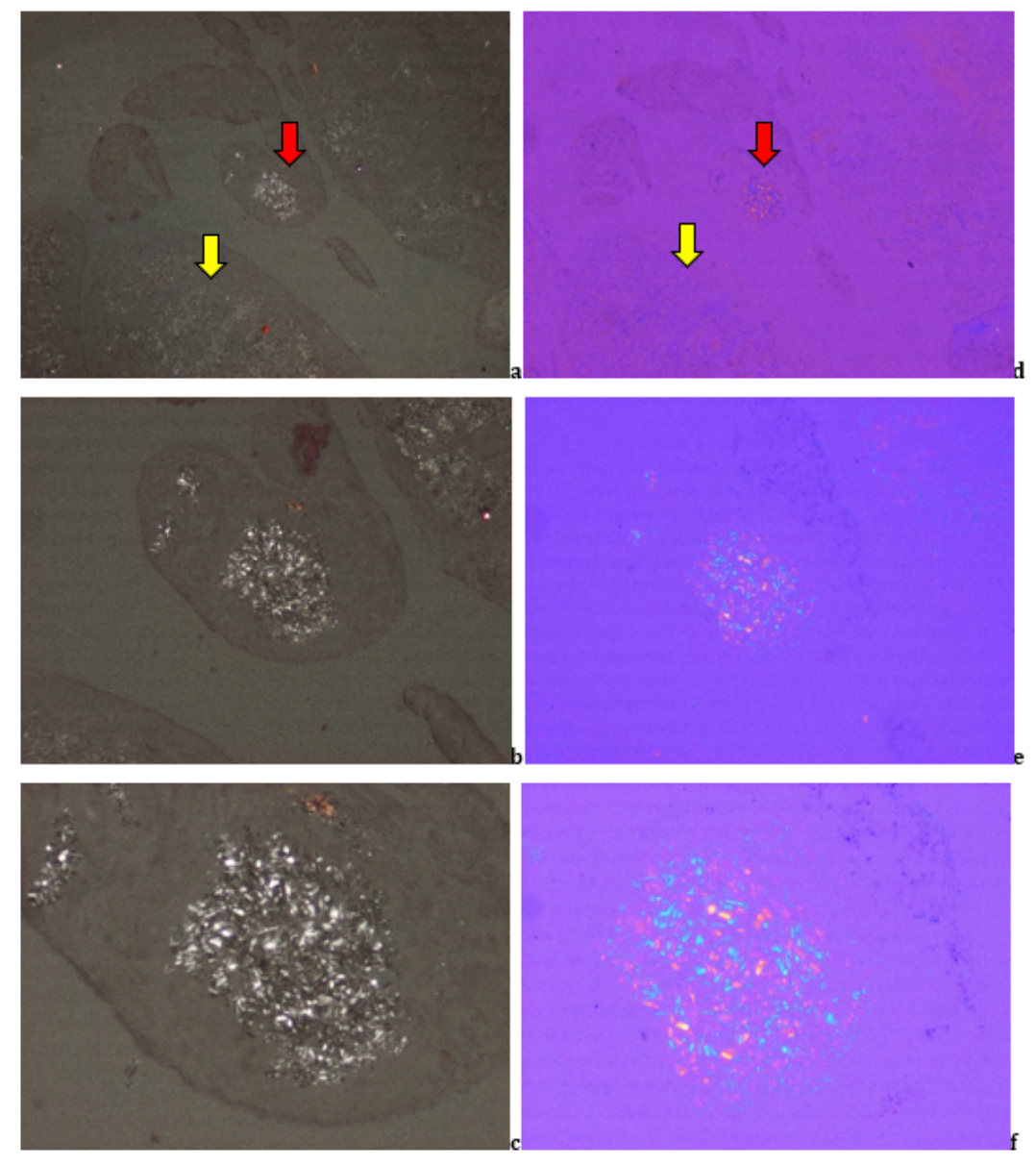

Figure 8a-f: *Synivial membrane, hydroxyapatite (HA) [Ca5(PO4)3(OH)] and CPPD [Ca2P2O7.2H2O] crystal deposits, unstained section, viewed under polarized light without Red I compensator: (a, b and c), and with Red I compensator: (d, e and f).*

The birefringence of the small HA crystal fragments is weak see yellow arrow head in Figures 8a and 8d), compared to the large, strongly positive birefringent CPPD crastals see red arrow head in Figures 8a and 8d). Under polarized light the direction of birefringence is positive according to the long axis of HA and CPPD crastals.

Unstained sections, viewed under polarized light: (a) x40, (b) x100, (c) x200

Unstained sections, viewed under polarized light, using Red I compensator (same fields): (d) x40, (e) x100, (f) x200

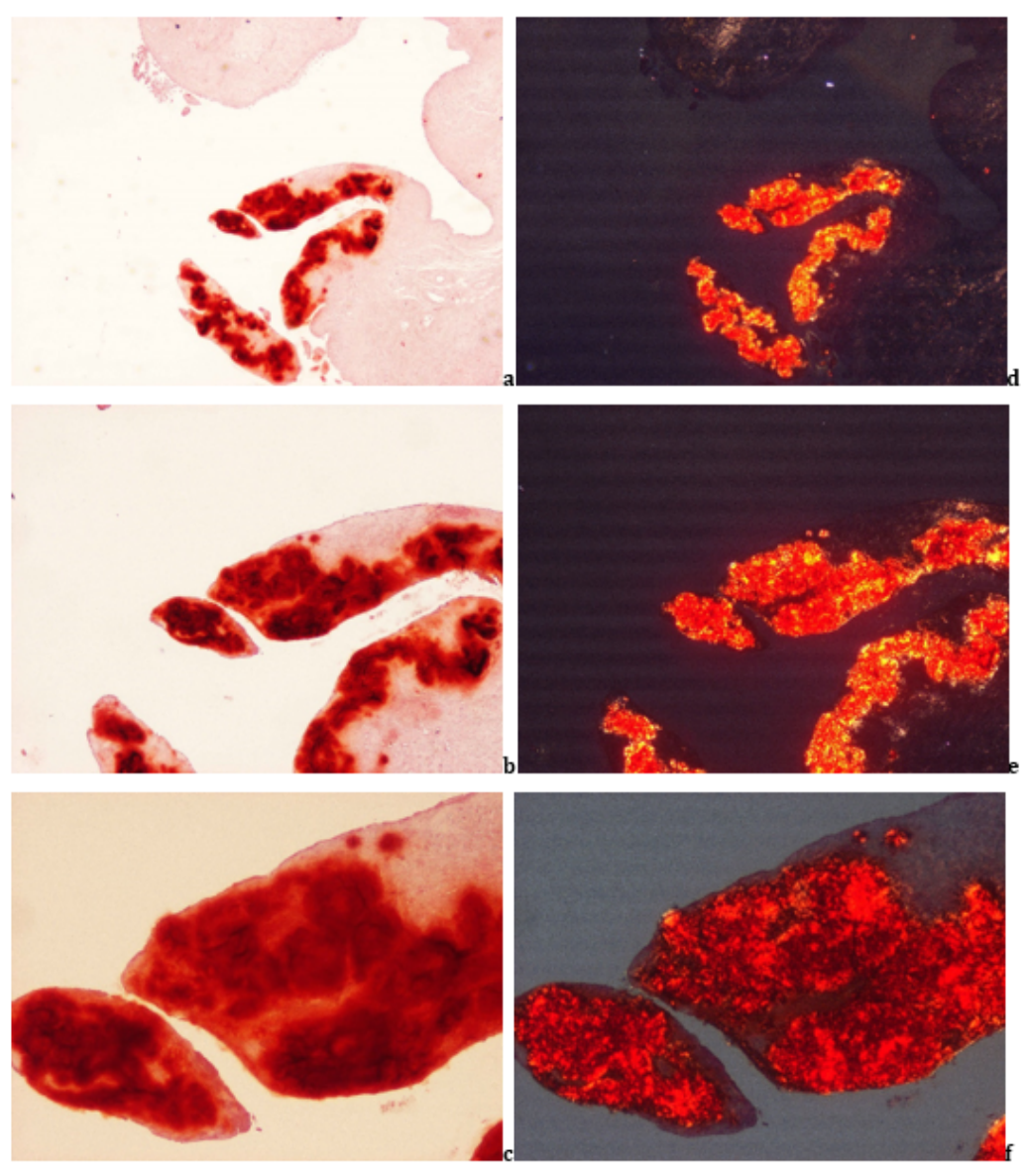

Figure 9a-f: *Synovial membrane, hydroxyapatite arthropathy, Alizarin red S staining (specific for calcium), viewed with the light microscope, and under polarized light, respectively.*

The HA and CPPD crystals are accompanied with non-crystalline (amorphous), calcium phosphate or calcium carbonate containing mineral deposits.

The HA crystals are readily soluble and are not detected in traditionally fixed tissue specimens stained with Alizarin red S.

The larger, partially masked birefringent crystals correspond to CPPD crystals, which occasionally may be detected in amorphous mineral deposits.

Alizarin red S, viewed with the light microscope: (a) x20, (b) x40, (c) x100

Alizarin red S, viewed under polarized light (same fields): (d) x20, (e) x40, (f) x100.

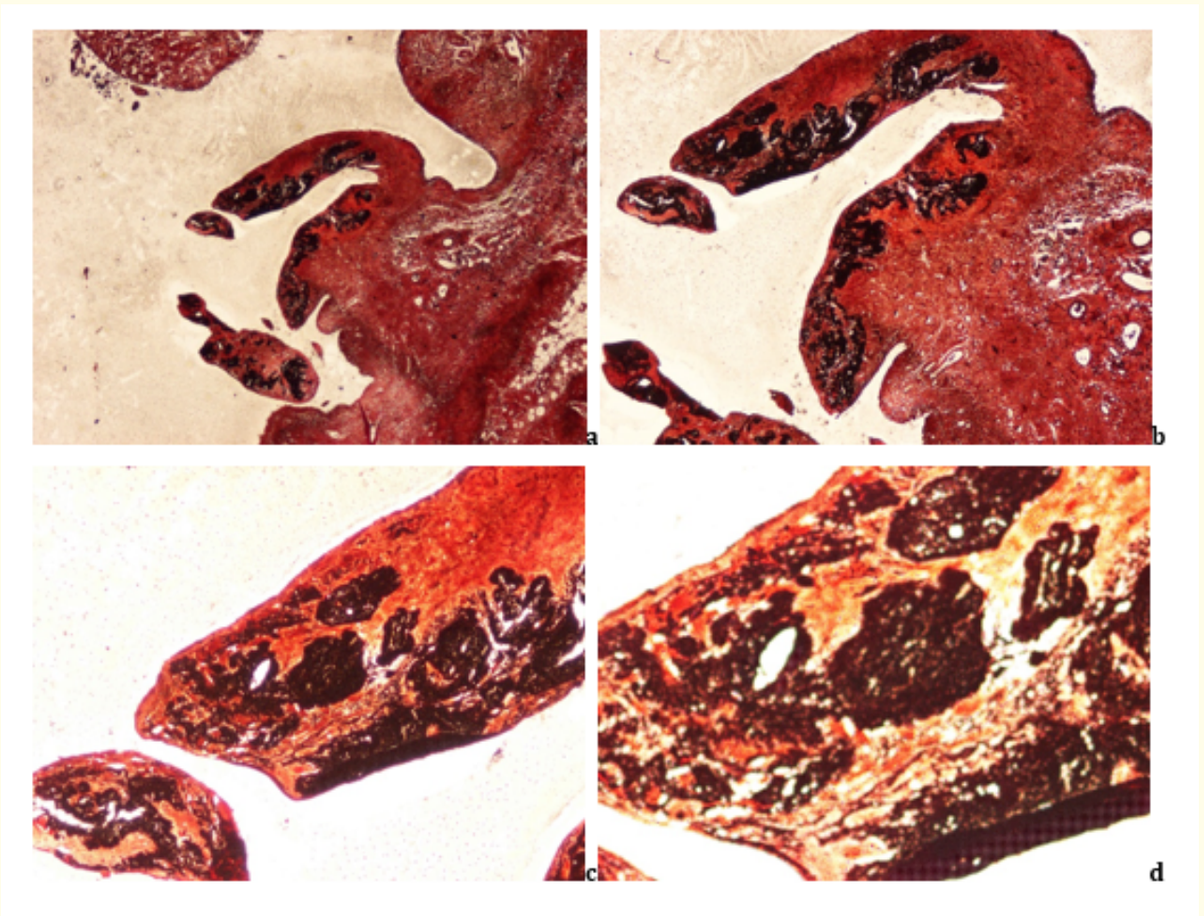

Figure 10a-d: *(same fields as in Figure 9a-f)*
Synovial membrane, hydroxyapatite arthropathy, von Kossa's reaction (specific for phosphate and/or carbonate), viewed with the light microscope.
The HA and CPPD crystals are accompanied by non-crystalline (amorphous), calcium phosphate containing mineral deposits.
The HA crystals are readily soluble and are not detected in traditionally fixed tissue specimens stained with von Kossa's reaction.
The CPPD crystals are partially masked by amorphous calcium phosphate or calcium carbonate deposits or appear as emty spaces (crystaline structures of calcium or phosphorus do not stain with Alizarin red S or the von Kossa reaction).
Von Kossa's reaction, viewed with the light microscope: (a) x20, (b) same as (a) x40, (c) same as (b) x100, (d) same as (c) x200.

Microscopic characteristics of clinically diagnosed chondrocalcinosis

(Calcium pyrophosphate dihydrate [$Ca_2P_2O_7.2H_2O$] **CPPD** crystals induced arthropathy).

In most cases **chondrocalcinosis** was characterized by amorphous calcium phosphate and/or calcium carbonate deposits of irregular shape in sections stained with HE, Alizarin red S or von Kossa's reaction, with or without demonstrable crystalline contents. Ocasionally the amorphous mineral contents were minimal (Figure 13-a-f).

CPPD crystals were less soluble in an 8%aqueous formaldehyde solution and water containing dyes than **HA** crystals, and more or less **CPPD** crystals were ocasionally preserved and demonstrated in conventionally fixed tissues stained by HE, Alizarind red S or the von Kossa reaction (lesser amounts of CPPD crystals dissolved during conventional fixation or histotechnical procedures in alcohol, acetone, xylene and in solutions of dyes).

In synovium, menisci or hyaline cartillage the inflammatory reaction around amorphous calcium phosphate and/or calcium carbonate mineral deposits was usually moderate or absent. The CPPD crystals with or without amorphous mineral deposits ocasionally have been accompanied with a cellular reaction including macrophages.

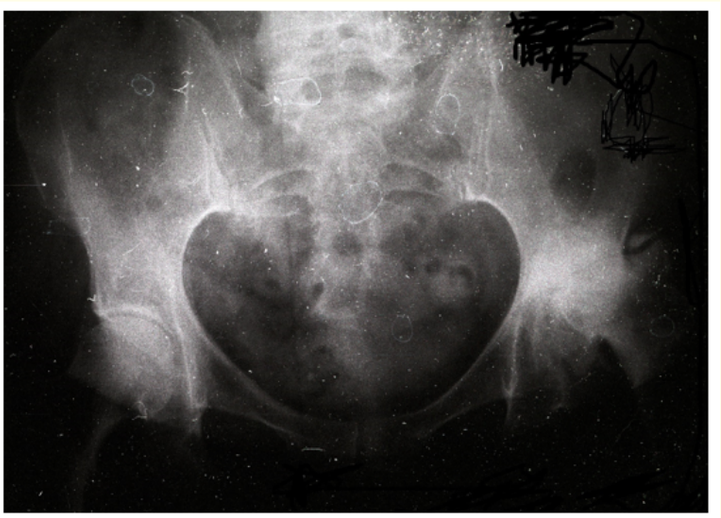

Figure 11: *Pre-operative pelvic antero-posterior x-ray.*
Destructive arthropathy of the left hip before total hip prosthesis implantation.

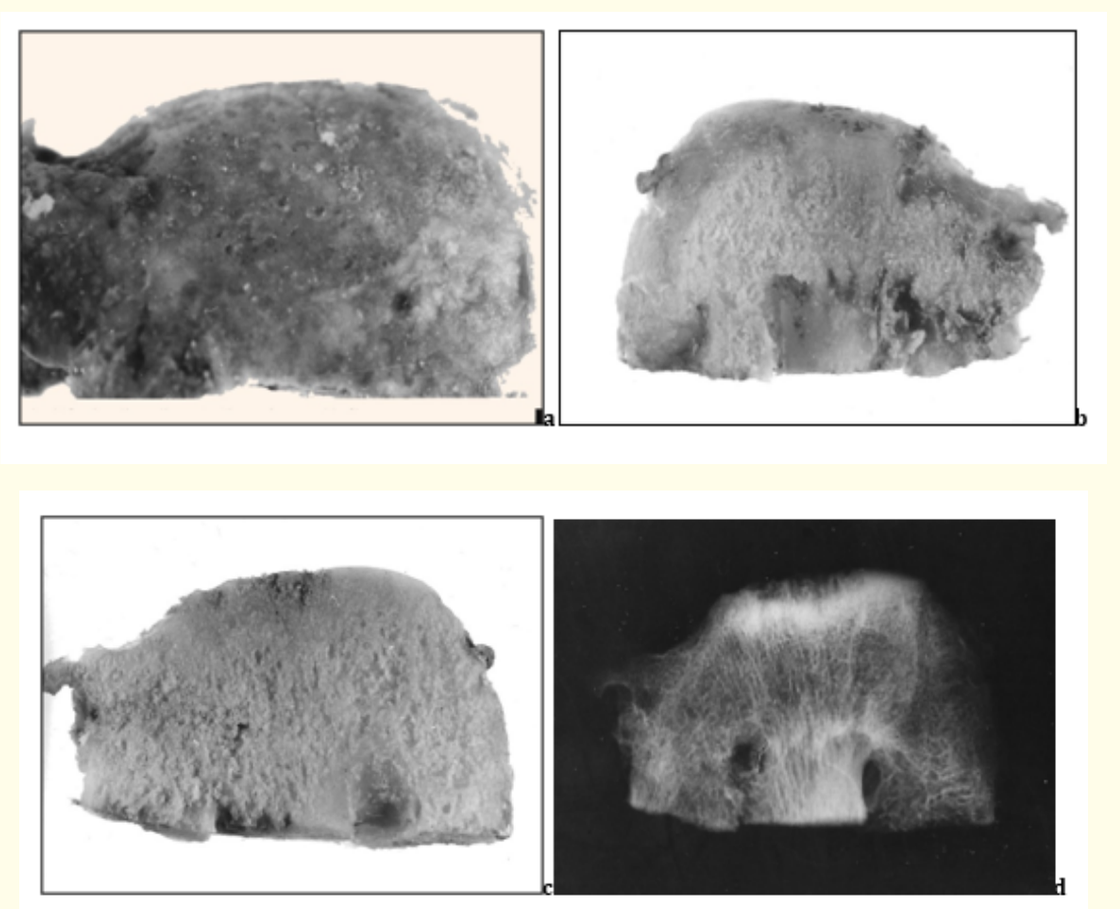

Figure 12a-d: *Surgucal specimen of the left femoral head, macrophotographs and contact X-Ray.*
(a) Destructive arthropathy, with scattered spotty mineral deposits on the surface of the left femoral head.
(b and c) Cut surfaces of the median and paramedian section of the left head of femur.
(d) Contact X-Ray of the median section of femoral head.

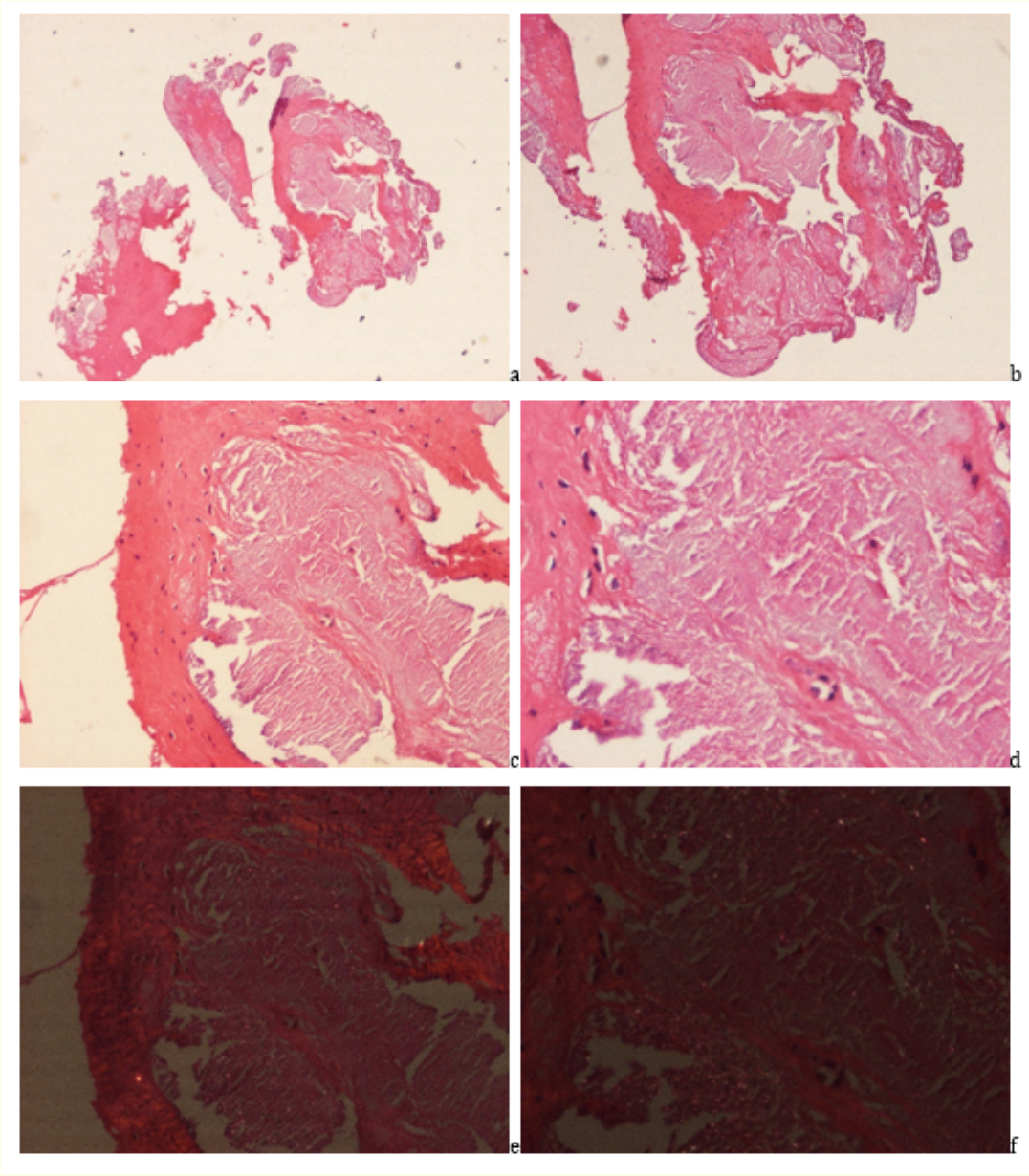

Figure 13a-f: *Chondrocalcinosis, HE, viewed with the light microscope (a-d), and viewed under polarized light (e-f).*
(a) Mineralisation of amorphous deposits is mild (moderate blue violet colour of amorphous calcium phosphate and/or carbonate), HE x20, (b) same as (a) x40, (c) same as (b) x100, (d) same as (c) x200.
CPPD crystals are less soluble than the HA crystals and may be retained in traditionally fixed and processed tissue sections, but small amounts of CPPD crystals dissolve, like HA crystals. In longer fixed tissues or in decalcinated bone crystals are usually not detected.
(e) CPPD or HA crystals are not detected in tissue sections stained by HE; same fields as (c) viewed under polarized light x100, (f) same fields as (d), viewed under polarized light x200.

Degenerated left hip joint with mineral deposits in a 75-year-old female patient (Pr n^0/y: 631/1980) with the clinical diagnosis of chondrocalcinosis are demonstrated by plain film radiography, macrophotographs and contact X-Ray in Figures 11 and 12.

In **unstained** sections according to Bély and Apáthy's non-staining techniques large amounts of **CPPD** crystals were detected in contrast to the moderate amount of **HA** crystals in **six** of 16 patients with clinically diagnosed chondrocalcinosis (Figure 14a-e).

In **five** of 16 patients with clinically diagnosed chondrocalcinosis, the **HA** crystals were dominant in contrast to **CPPD** crystals, and in further **five** of 16 patients more or less **CPPD** crystal deposits accompanied more or less **HA** crystals.

The **CPPD** crystals had a rhomboid shape, ranged in size from 5 to 40µm (Figures 14a-e and 15a-e), and showed a strong positive (Figure 15a-e) birefringence, in contrast to the weak birefringence of **HA** crystals.

Figures 14a-e and 15a-e show **CPPD** and **HA** crystals deposits side by side in the same tissue sections; the birefringence of **CPPD** crystals is strong compared to the weak birefringence of **HA** crystals (Figures 14a-e). The birefringence of **CPPD** and **HA** crystals is positive according to their long axis, like that of collagen fibers (Pr n^0/y: 1558/2002).

Figures 16a-b and 17a-b show characteristic amorphous calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits, stained with Alizarin red S and by von Kossa's reaction, with the clinical diagnosis of chondrocalcinosis (Pr n^0/y: 2086/1994).

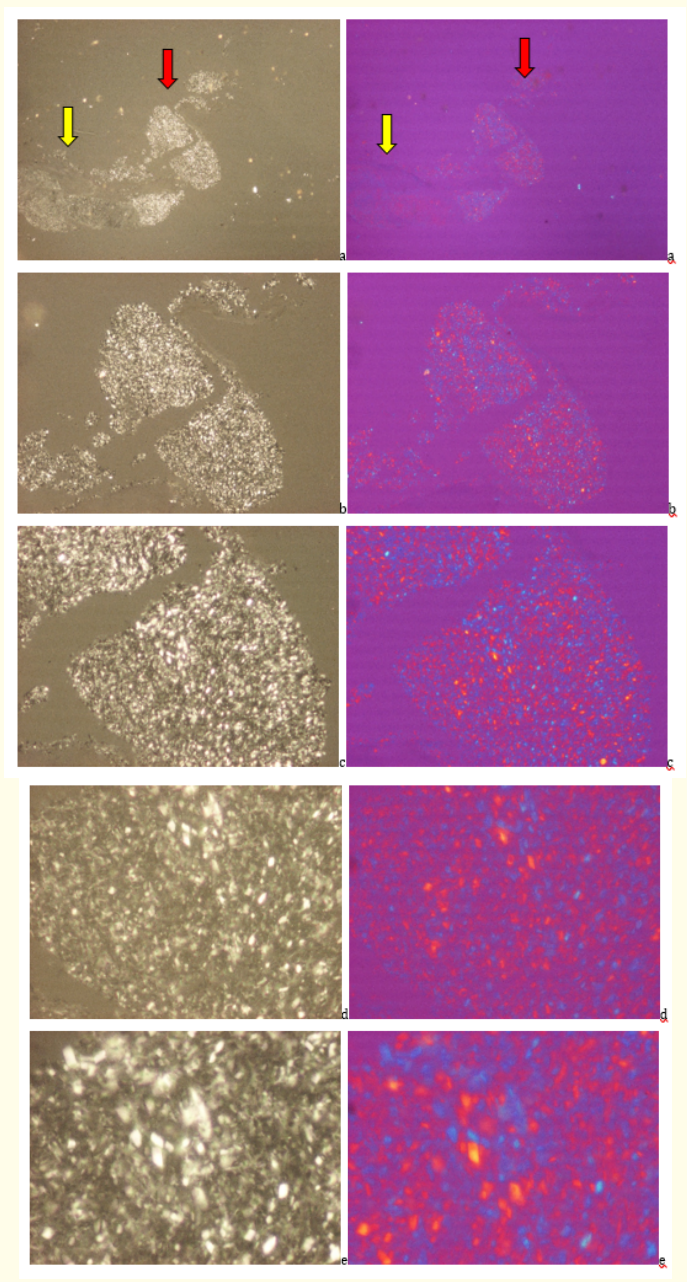

Figure 14a-e and Figure 15a-e: *Hydroxyapatite arthropathy, HA crystals, and CPPD crystal deposits, side by side in the same unstained tissue section.*

The HA crystals are colorless, translucent, small (50-500 nm), rod-shaped prisms or fragments, and form microaggregates. Under polarized light the intensity of birefringence of HA crystals is weak and positive (see yellow arrow head in Figures 14a and 15b).

The large groups of CPPD crystals are also present, with a similarly positive but stronger birefringence (see red arrow head in Figures 14a and 15b).

(14a-e) unstained section, viewed under polarized light, without Red I compensator, (a) x40, (b) x100, (c) x200, (d) x400, (e) x600

(15a-e) unstained section, viewed under polarized light, with Red I compensator, same fields as 14 a-e.

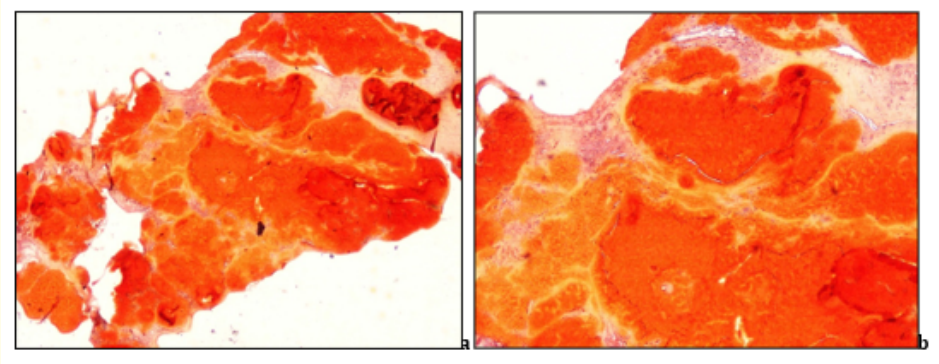

Figure 16a-b: *Clinically diagnosed chondrocalcinosis, synovial membrane, Alizarin red S staining viewed under the light microscope. The HA and CPPD crystals are accompanied by non-crystalline (amorphous) deposits, containing calcium phosphate or calcium carbonate The HA crystals are readily soluble and are not detected in traditionally fixed tissue specimens stained with Alizarin red S. Alizarin red S-stained synovia viewed under the light microscope: (a) x20, (b) same as (a) x40.*

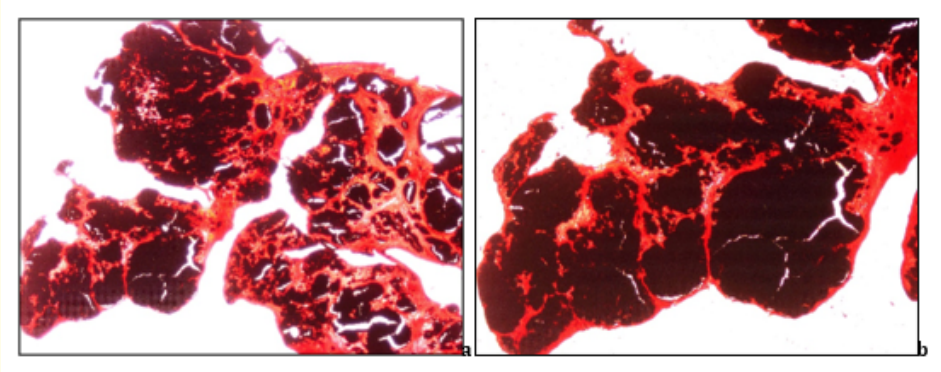

Figure 17a-b: *(same as 16a-b)*
Clinically diagnosed chondrocalcinosis, synovial membrane, von Kossa' reaction (specific for carbonate or phosphate), viewed under the light microscope.
The CPPD crystals are masked by amorphous calcium phosphate or calcium carbonate deposits. Von Kossa's reaction, viewed under the light microscope: (a) x20, (b) same as (a) x40.

Table 1 summarizes the histological characteristics of **HA** and/or **CPPD** crystals, furthermore amorphous calcium phosphate or carbonate deposits of patients with clinically diagnosed apatite rheumatism and chondrocalcinosis.

Clinical diagnosis	HA [$Ca_5(PO_4)_3(OH)$] crystal deposits	CPPD [$Ca_2P_2O_7.2H_2O$] crystal deposits	[$CaPO_4$] or [$CaCO_3$] stained by HE, Alizarin red S or von Kossa reaction
Apatite rheumatism			
3495/97* (right knee) prepatellar bursa	**Dominant** Clusters and aggregates	Sporadic	Positive, abundant
3496/97* (left knee) prepatellar bursa	**Dominant** Clusters and aggregates	Sporadic	Positive, abundant
4443-1997 (right knee) synovial membrane	**Dominant** Clusters and aggregates	Scattered focal clumps in toto less than 10%	Positive, abundant
C17-2012 (right knee) Synovial punctatum	**Dominant**	Sporadic	Positive
2417-2014 (right hip) synovial membrane	60% Clusters and aggregates	40% Scattered focal clumps	Positive, abundant
604-2015 (right knee) synovial membrane	60% Clusters and aggregates	40% Scattered focal clumps	Positive, abundant
4V/2170-19 (right knee) synovial membrane, capsule, bone	**Dominant** Clusters	Sporadic	Negative
V/2171-19 (right shoulder) synovial membrane, capsule, bone	**Dominant** Clusters	Sporadic	Negative
181-2019 (both shoulders) Synovial punctatum	**Dominant**	Sporadic	Positive
Chondrocalcinosis			
14-1975 (right hand, wrist) tendon sheats	Sporadic	**Dominant**, focal clumps of fragmented crystals	Positive, abundant
2560-1978 (shoulder) synovial membrane, capsule, bone	**Dominant**	Sporadic	Positive
631-1980 (left hip) synovial membrane, capsule, bone	Sporadic	**Dominant**	ND
676-1981 (ND) synovial membrane, capsule, bursa	**Dominant**	Sporadic	Positive, abundant
2207-1981 (ND) synovial membrane, capsule	**Dominant**	Sporadic	Positive
834-1983 (hip) synovial membrane, bursa, tendon	**Dominant**	Sporadic	Positive, abundant
2739-1983 (hip) synovial membrane, capsule, bone	Sporadic	**Dominant**	Positive, abundant
3097-1985 (left knee) synovial membrane, capsul, bone	**Dominant**	Sporadic	Positive, abundant
437-1986 (left hip) synovial membrane, capsule	Sporadic	**Dominant**	Positive, abundant
3439-1987 (knee) synovial membrane, capsule	**Mixed HA-CPPD** disease; **HA:70 %**	**Mixed HA-CPPD** disease; **CPPD:30 %**	Positive, abundant
192-1989 (wrist) synovial membrane	**Mixed HA-CPPD** disease; **HA:40 %**	**Mixed HA-CPPD** disease; **CPPD:60 %**	Positive, abundant
2086-1994 (left knee) synovial membrane	**HA varies per foci 10- 90 %**	**Dominant CPPD** Varies per foci**: 10-90%**	Positive, abundant
3558-2002 (left knee) synovial membrane, capsule	Sporadic	**Dominant** (in some foci only fragments)	Positive, abundant
169-2006 (left wrist) synovial membrane, capsule	**Mixed HA-CPPD** disease; **HA:80 %**	**Mixed HA-CPPD** disease; **CPPD:20 %**	Positive, abundant
581-2007 (right knee) synovial membrane	**Mixed HA-CPPD** disease; **HA:60 %**	**Mixed HA-CPPD** disease; **CPPD:40 %**	Positive, abundant
477-2008 (right knee) synovial membrane, capsule, bursa	**Mixed HA-CPPD** disease; **HA:60 %**	**Mixed HA-CPPD** disease; **CPPD:40 %**	Positive

Table 1: *Histological characteristics of HA and CPPD crystals, furthermore amorphous calcium phosphate or carbonate deposits in apatite rheumatism and chondrocalcinosis.*

Remark to Table 3

Apatite rheumatism and chondrocalcinosis were diagnosed clinically.

Calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits were stained with HE, Alizarin red S staining or von Kossa reaction.

Calcium phosphate [$CaPO_4$] and/or calcium carbonate [$CaCO_3$] deposits may mask the occasionally retained **CPPD** or exceptionally preserved **HA** crystals in tissue sections.

The crystalline structure of calcium or phosphate does not stain with HE, Alizarin red S staining or von Kossa reaction, the **HA** or **CPPD** crystals remain intact by conventional stainings and reaction.

HA [$Ca_5(PO_4)_3(OH)$] or **CPPD** [$Ca_2P_2O_7.2H_2O$] crystal deposits were demonstrated with the non-staining techniques of Bély and Apáthy (2013).

*In two cases the **HA** [$Ca_5(PO_4)_3(OH)$] crystals were identified by electron diffraction as well.

The chemical characteristics of hydroxyapatite are not uniform in the pertinent literature; the formula of **HA** is [$Ca_5(PO_4)_3(OH)$] or [$Ca_{10}(PO_4)_6OH_2$].

The size of individual **HA** crystals is submicroscopic [21], according to others (50-500 nm) [22], in clusters (1-5 μm) [22] or (1.9-15.6 μm) [23].

In apatite rheumatism the **HA** crystal clusters "tend to aggregate into globular clumps, and can appear as refractile shiny coins on light microscopy, but show no birefringence under compensated polarized microscopy (400 x)" by traditional stains [9].

Using a professional polarizing microscope with high, at least 100-Watt brightness the **HA** crystal clusters (1-5 μm) are visible and birefringent with polarized light (100x), moreover they can form larger aggregates (conglomerates) of 100 μm size or larger, which are visible with an objective of 40x by non-staining techniques [14, 15] (see Figure 14a).

The birefringence of **HA** crystals is weak compared to the strong birefringence of **CPPD** crystals.

"Dominant" means that more than 90% of crystals are **HA** or **CPPD**

"Sporadic" means, that less than 10% of crystals are **HA** or **CPPD**

Amounts of **HA** and **CPPD** may vary per foci side by side within one tissue section.

CPPD crystals are less soluble in 8%aqueous formaldehyde solution and in aqueous solutions of dyes than **HA** crystals; variable amounts of **CPPD** crystals can be ocasionally preserved in tissue sections, and remain demonstrable in conventionally fixed tissues stained with HE, Alizarind red S or von Kossa's reaction.

In **unstained** sections viewed under polarized light the **CPPD** crystals have a rhomboid shape (Figures 11a-d and 14a-b). The expected size range is 0.42-17.9 μm [21] or according to others it varies from submicroscopic to 40μm [23].

With Red I compensator the **CPPD** crystals show a strong (Figure 14a-e) positive (Figure 15a-e) birefringence, in contrast to the weak (Figure 14a-e) positive (Figure 15a-e) birefringence of **HA** crystals.

ND – No Data

Discussion

The clinical symptoms of apatite rheumatism and chondrocalcinosis are similar with overlapse of the most commonly involved joints.

Apatite rheumatism is characterized by sudden onset of severe pain, swelling, tenderness, and restricted motion of joints, with overlying redness and warmth [9]; pain on use, large cool viscous effusions, instability and rapid progression may occur [24]. Intra-articular **HA** crystal deposits can cause joint destruction [25].

Not all patients are symptomatic [25]; the asymptomatic cases are most commonly discovered as an incidental finding on plain film radiography [9].

According to the pertinent literature the shoulder (rotator cuff) is most commonly affected [9, 24], but deposits can occur around almost any joints (hip, spine, knee, elbow, wrist, ankle joint, etc.) [9, 25, 26].

In our patients group the knees were the most commonly involved joint.

Chondrocalcinosis is a metabolic arthropathy caused by **CPPD** crystal deposition in and around joints (synovium, capsules, tendons and ligaments) [26]. Calcium pyrophosphate deposition is often asymptomatic, but the crystal deposits can also cause joint damage; the affected joints are usually swollen, warm, and severely painful [27].

Almost any joint may be involved by **CPDD**, although the knees, wrists, and hips are most often affected [26, 27].

In our patients, in agreement with pertinent literature, the knees were the most common localization of chondrocalcinosis.

At present the possible treatment of apatite rheumatism and chondrocalcinosis is similar [28].

Treatment of apatite rheumatism usually requires analgesics, with or without aspiration of the calcific deposits, and at times even open surgery is necessary for relief of pain [25].

In cases of chondrocalcinosis analgetic non-steroidal anti-inflammatory drugs may be useful to a limited extent, and deposits may be removed by arthroscopic or open surgery as well.

The use of steroids is recommended only as a second line treatment [28].

Our results suggest that the clinical categories for different metabolic disorders should be based on the quality of dominant crystals in deposits, rather than localization or clinical symptoms, which are overlapping. In other words, different metabolic diseases should be categorized based on the presence of dominant crystals and not on the overlapping localization or clinical symptoms.

In both disease crystals accumulate in the synovial membranes, in articular hyaline and/or fibrocartilage, joint capsules, bursae, tendon sheaths and in periarticular connective tissue with or without calcification.

In our patient groups apatite rheumatism was characterized by the dominance of **HA** crystals in contrast to **CPPD**, accompanied with abundant calcium phosphate or carbonate deposits.

In chondrocalcinosis the abundant calcium phosphate or carbonate deposits were associated with a dominant **CPPD** crystal content, in contrast to **HA** crystals.

In some cases of aptite rheumatism or chondrocalcinosis minimal metaplastic cartillage formation was detected in synovial membranes.

According to Resnick (1988) when cartilage calcification is widespread, **CPPD** arthropathy should be considered. Diffuse, amorphous calcifications within the joint are more likely to be due to hydroxyapatite deposition disease. The distinction between these entities is further complicated by the fact that the two may coexist as a mixed calcium phosphate crystal deposition disease. [29].

Using Bély and Apáthy's non-staining technique (2013) different amount of **CPPD** and **HA** crystals were demonstrated in all of our patients with the clinical diagnosis of apatite rheumatism or chondrocalcinosis (Table 1).

In **four** of five patients with the clinical diagnosis of apatite rheumatism large amounts of intrasynovial **HA** crystal were present, supporting Resnick's opinion [29].

Retrospectively, in one (2417/2014) of five patients with clinically diagnosed apatite rheumatism the original diagnosis had to be corrected as coexistent (mixed) apatite rheumatism and chondrocalcinosis, based on the nearly same amount of **HA** (60%) and **CPPD** (40%) crystals in unstained sections viewed under polarized light (Table 1).

The original clinical diagnosis of chondrocalcinosis was confirmed in **6** of 16 patients, by the huge amount of **CPPD** crystals and moderate amount of **HA** crystals in unstained sections viewed under polarized light.

The original clinical diagnosis of chondrocalcinosis had to be corrected and recognized as apatite rheumatism in **5** patients, because of large deposits of **HA** with moderate amounts of **CPPD** crystals.

Retrospectively, **5** of 16 patients shoud be regarded to have coexistent (mixed) **HA** and **CPPD** crystal induced metabolic diseases, based on the ratio of **HA** and **CPPD** crystals (Table 1).

Apatite rheumatism and chondrocalcinosis are progressive crystal deposition diseases, characterized in their early stage only by a few small, and later on more and larger, sometimes confluent deposits.

In our patients more or less **HA** or **CPPD** crystals existed side by side in crystal deposits. **HA** crystal deposits were always accompanied with scattered or more or less amount of **CPPD** crystals, and **CPPD** crystal deposits were not detected without **HA** crystals

Crystal deposition seems to be starting with **HA** crystal deposition, followed later by **CPPD** deposition. **HA** crystal deposition may be accompanied with an intensive inflammatory reaction and phagocytosis (Figure 7a-f).

Amorphous calcium phosphate or carbonate deposition are a late phenomenon and are detected only in advanced stages with HE staining as blue-violet foci. Amorphous mineral deposits are without inflammatory reactions.

This histologically outlined progressive process is reflected in the clinically recommended stages of crystal-induced diseases; for chondrocalcinosis: asymptomatic CPPD disease ("asymptomatic CPPD"), acute CPP crystal arthritis ("pseudogout"), and chronic CPP crystal inflammatory arthritis ("pseudo-RA") were recommended [30], and for apatite rheumatism: precalcific, formative, resting phases,including resoptive phase characterized by inflammation, and postcalcific phase characterized by reparative processes [31].

The correction of the clinical diagnosis of chondrocalcinosis according to our findings has to be explained.

In conventionally stained tissue sections viewed under polarized light only **CPPD** crystals are visible, **HA** crystals are not.

Dominant **CPPD** crystals with strong birefringence can suppress the weakly birefringent **HA** crystals, which are detected only with non-staining technique.

Reducing the intensity of microscopic light viewing not stained sections, only the stark birefringent **CPPD** crystals were detected, the weak birefringent **HA** crystals were not, which may lead to the incorrect diagnosis of chondrocalcinosis.

For the precise analysis of crystal deposits, a professional polarizing microscope is needed, with high, at least 100-Watt brightness. Using a less reliable polarizing microscope with ineffective brightness, weak birefringent **HA** crytals can be missed (remain undetected), leading to the uncorrect diagnosis of chondrocalcinosis.

The crystals may be best preserved in unstained frozen sections, without using conventional fixatives (aqueous formaldehyde solution), acetone, xylene and solutions of dyes. In frozen sections viewed under polarized light a large amounts of cholesterol crystals may be detected [32]. Unfortunately, in frozen sections large amounts of cholesterol crystals can obscure other crystals and the targeted crystals may be hidden. The use of fat solvents can be useful to identify other crystals than cholesterol, that are less soluble in fat solvents.

With the non-staining technique of Bély and Apáthy several other crtystals may be detected; for exact identification of these electron microscopy, electron diffraction analysis or other methods are needed.

Conclusions

Our study indicates that apatite rheumatism and chondrocalcinosis are crystal induced diseases caused by the same basic metabolic disorder.

The diagnosis of these diseases should be based on the quality of the dominant crystals in the deposits and not according to the overlapping clinical symptoms or localization.

Assuming, that the apatite rheumatism and chondrocalcinosis are progressive crystal deposition diseases, it seems to be more likely, that they are different stages of the same metabolic disease, rather than two distinct entities.

The early stage with dominant **HA** crystal deposition is called "apatite rheumatism", and the advanced stage with dominant **CPPD** deposition "chondrocalcinosis".

The **HA** and/or **CPPD** crystals may provoke an intensive inflammatory reaction. In the late stage of the disease variable amounts of amorphous calcium phosphate and/or carbonate deposits enclose the crystals, thereby reducing or eliminating the inflammatory reaction.

Distinction between the two diseases seems to be of theoretical significance given present therapeutic possibilities.

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