ECRONICON OPEN ACCESS

EC CARDIOLOGY
Research Article

Crystal Induced Arthropathies - Retrospective Analysis of Synovial Fluids Aspirated Between 2005 and 2017

Ágnes Apáthy[1] and Miklós Bély[2]*

[1]*Department of Rheumatology, St. Margaret Clinic, Budapest, Hungary*

[2]*Department of Pathology, Hospital of the Order of the Brothers of Saint John of God in Budapest, Hungary*

***Corresponding Author:** Miklós Bély, Department of Pathology, Hospital of the Order of the Brothers of Saint John of God in Budapest, Hungary.

Received: December 03, 2022; **Published:** December 21, 2022

Abstract

Introduction: The hospital of the Order of the brothers of Saint John of God in Budapest is one of the main centers of a nationwide network of departments of rheumatology associated with a general hospital.

Symptomatic joints are regularly aspirated in our hospital.

Aim of the Study: The aim of this study was to determine the prevalence of various crystals in such synovial fluids.

Patient Population and Methods: In our hospital symptomatic joints of 2971 patients were aspirated between 2005 and 2017.

Technique of puncture, wet preparation and analysis of synovial fluids (including the speed of processing) followed generally accepted rules.

Results: The 2971 punctures of 279 patients revealed crystals in 294 samples; MSU was present in 87 (2.93%), CPPD in 180 (6.06%), HA in 4 (0.13%), cholesterol in 12 (0.40%), lipid liquid crystals in 10 (0.34%), and calcium oxalate in 1 (0.03%) of 294 cases.

The mean age of patients with cholesterol or MSU crystals was low compared to the mean age of patients with CPPD or HA crystals.

None of our patients suffered of manifest metabolic maladies or crystal induced metabolic disorders, and arthropathies were not diagnosed in any of them.

Discussion and Conclusion: Crystals may be present in the synovial fluid of patients without clinically diagnosed metabolic diseases or arthropathies.

The fast processing of synovial fluids, the exact technical circumstances, furthermore the use of a professional polarizing microscope with high, at least 100-Watt brightness are crucial for a correct crystal analysis.

Keywords: *Synovial Fluid; Crystal Analysis*

Abbreviations

HE: Hematoxylin Eosin; MSU: Monosodium Urate Monohydrate [$NaC_5H_3N_4O_3 \cdot H_2O$] (Monosodium Salt of Uric Acid [$C_5H_4N_4O_3$]; CPPD: Calcium Pyrophosphate Dehydrate - [$Ca_2P_2O_7.2H_2O$]; HA: Calcium Hydroxyapatite - [$Ca_5(PO_4)_3(OH)$]; Cholesterol: [$C_{27}H_{46}O$]; Lipid Liquid Crystals; Calcium Oxalate: [CaC_2O_4]; Pr n^0/y: Protocol Number/Year

Introduction

Crystal induced arthropathies such as gout, apatite rheumatism, chondrocalcinosis etc. are characterized by various crystals in the synovial fluid with or without crystal deposits in articular and periarticular tissues (synovial membrane, capsule, bursae, tendon sheaths, articular cartilage, bone).

A wide spectrum of clinical symptoms may accompany the crystal induced arthropathies: sudden onset of severe pain, intraarticular fluid accumulation, swelling, stiffness, tenderness, limited motion of affected joint with redness and warmth of overlying skin, rapid progression of joint destruction and instability, creaking, popping noise during movement or locking of the joint [1-5].

The metabolic disturbances are not always accompanied with clinical symptoms; indeed, numerous metabolic disorders may be asymptomatic [6-10].

Aim of the Study

The aim of this study was to determine the prevalence of various crystals in synovial punctures.

Patient Population and Methods

The hospital of the Order of the brothers of Saint John of God in Budapest is one of the main centers of a nationwide network of departments of rheumatology associated with a general hospital.

Symptomatic joints are regularly aspirated in our hospital.

We present the results of our study of the crystalline contents of synovial fluids between 2005 and 2017.

Cytology, laboratory analysis (chemistry) of synovial fluid, infective agents, remnants of intraarticular steroid injections, foreign bodies, metallic or methylmetacrylate fragments etc. are not the topic of this study.

Technique of puncture, wet preparation and analysis of synovial fluids (including the processing speed) were according to the generally accepted rules [11].

Spontaneous coagulation of the synovial fluid was inhibited with sodium heparin in cases where analysis was not possible within a short period of time.

The lithium heparin or calcium oxalate anticoagulants were avoided, since they may produce crystalline artefacts [11].

Only those synovial fluids were considered pathogenic for MSU and CPPD or HA crystals, in which intracellular (phagocytosed) crystals were also present.

Unstained synovial fluids were examined for crystals with the light microscope (Olympus BX51) and under polarized light, respectively.

Selected cases of synovial fluids were stained with HE [12], Alizarin red S [13,14] or Oil Red O [15].

In selected cases the crystals were identified with a JEM 100CX electron microscope and electron diffraction as well.

Demographics of female and male patients were compared with the student (Welch) t-probe [16].

Results

Two hundred ninety-four (294) of 2971 aspirates revealed various crystals in 279 patients; MSU was observed in 87 (2.93%), CPPD in 180 (6.06%), HA in 4 (0.13%), cholesterol in 12 (0.40%), lipid liquid crystals in 10 (0.34%), and calcium oxalate in 1 (0.03%) of 294 cases.

The presence of bipyramidal calcium oxalate crystals was exceptional, and was found only in 1 patient, this was regarded as an artefact due to the calcium oxalate anticoagulant.

Bilateral symptomatic knee joints were punctured at the same time in 8 patients; in all cases the same crystals were found in the simultaneously punctured knees.

The same joint was punctured repeatedly in 10 patients during the same period of hospitalization (8 joints twice, and 2 joints three times); in these joints' fluid accumulated repeatedly. The crystals were identical with the previous ones in these joints.

In 15 punctures of 14 patients two types of crystals were found in the same synovial fluid:

- MSU and CPPD crystals were identified in 5 synovial fluids of 4 patients at the same time,
- CPPD and HA crystals were found in 4 patients,
- CPPD and cholesterol crystals were present in 4 patients, and
- CPPD and lipid liquid crystals were observed in 2 patients.

Table 1 summarizes these observations.

Year of puncture	N of puncture	MSU	CPPD	HA	Cholesterol	Lipid	Oxalate	N of crystals	in % of puncture/ year
2005	284	18	15	0	0	3	0	36	12,68
2006	283	15	4	0	0	3	0	22	7,77
2007	193	6	4	0	1	2	0	13	6,74
2008	145	8	14	1	0	1	0	24	16,55
2009	262	4	24	0	0	1	1	30	11,45
2010	201	3	22	1	1	0	0	27	13,43
2011	244	10	33	0	1	0	0	44	18,03
2012	282	4	15	0	4	0	0	23	8,16
2013	256	5	21	0	3	0	0	29	11,33
2014	226	5	7	0	1	0	0	13	5,75
2015	171	4	3	1	0	0	0	8	4,68
2016	229	0	11	1	0	0	0	12	5,24
2017	195	5	7	0	1	0	0	13	6,67
Total	2971	87	180	4	12	10	1	294	9,896
In %	100%	2,93	6,06	0,13	0,40	0,34	0,03	9,896	

Table 1: *Synovial fluids with crystals between 2005 and 2017.*

Key to table 1: MSU: Monosodium Urate Monohydrate [$NaC_5H_3N_4O_3 \cdot \boldsymbol{H}_2O$] (Monosodium Salt of Uric Acid [$C_5H_4N_4O_3$]; CPPD: Calcium Pyrophosphate Dehydrate - [$Ca_2P_2O_7 2H_2O$]; HA: Calcium Hydroxyapatite - [$Ca_5(PO_4)_3(OH)$]; Cholesterol: [$C_{27}H_{46}O$]; Lipid Liquid Crystals; Calcium Oxalate: [CaC_2O_4]; Pr n^0/y: Protocol Number/Year.

The mean age of patients with MSU crystals in synovial fluids was low at the time of puncture compared to the mean age of the patients with the CPPD crystals (56.41 year versus 61.42 year; $p < 0.0024$) or compared to the mean age of patients with HA crystals (56.41 year versus 70,.75 year; $p < 0.0346$); these differences were significant (Table 2 and 3).

The mean age of patients with cholesterol crystals in synovial fluids was low at the time of puncture compared to the mean age of the patients with CPPD crystals (52.67 year versus 61.42 year; $p < 0.0461$) or compared to the mean age of patients with HA crystals (52.67 year versus 70.75 year; $p < 0.0128$); these differences were also significant (Table 2 and 3).

Comparing the average age of men and women, we found no significant difference between the individual crystal groups (Table 2 and 3).

There was also no significant difference in the average age of men and women within the same crystal groups (Table 2).

Table 2 summarizes the patients' demographics with MSU (n = 87), CPPD (n = 180), HA (n = 4), cholesterol (n = 12) and lipid liquid crystals (n = 10).

Number of patients with various crystals	Total n of crystals* (n = 294)	Mean age in years at puncture ± SD	Range of age (In years)
Total number of patients (n = 279*)	**279 of 294**	**59.50 ± 13.92**	**19 - 93**
Female	119	62.86 ± 13.91	19 - 93
Male	160	57.00 ± 13.44	19 - 91
Patients with MSU crystals (n = 87)	**87 of 294**	**56.41 ± 11.39**	**29 - 91**
Female	10	62.60 ± 9.23	49 - 79
Male	77	55.61 ± 11.45	29 - 91
Patients with CPPD crystals (n = 180)	**180 of 294**	**61.42 ± 14.50**	**19 - 93**
Female	101	63.49 ± 13.95	23 - 93
Male	79	58.77 ± 14.84	19 - 87
Patients with HA crystals (n = 4)	**4 of 294**	**70.75 ± 8.54**	**60 - 79**
Female	2	72.00 ± 5.66	68 - 76
Male	2	69.50 ± 13.44	60 - 79
Patients with Cholesterol crystals (n = 12)	**12 of 294**	**52.67 ± 13.21**	**19 - 71**
Female	5	49.80 ± 19.07	19 - 71
Male	**7**	54.71 ± 8.12	38 - 63
Patients with Lipid liquid crystals (n = 10)	**10 of 294**	**59.70 ± 11.39**	**42 - 80**
Female	6	60.67 ± 12.23	42 - 80
Male	4	58.25 ± 11.64	49 - 75

Table 2: *Sex, mean age with SD and range (in years) of 294 patients with various crystals of synovial fluids.*

*Remark to table 2: *In 15 synovial fluids two crystals were identified (279+15 = 294 crystals).*

Table 3 summarizes the differences in mean age of patients with MSU (n=87), CPPD (n=180), HA (n=4), cholesterol (n=12) and lipid liquid crystals (n=10).

p < 0.05 at an alpha level of 0.05	Age
With MSU n = 87 versus with CPPD n = 180 of 294	**0,0024**
Female n = 10 of 87 versus female n = 101 of 180	0,7882
Male n = 77 of 87 versus male n = 79 of 180	0,1377
With MSU n = 87 versus with HA n = 4 of 294	**0,0386**
Female n = 10 of 87 versus female n = 2 of 4	0,1823
Male n = 77 of 87 versus male n = 2 of 4	0,3783
With MSU n = 87 versus with Cholesterol n = 12 of 294	**0,3661**
Female n = 10 of 87 versus female n = 5 of 12	0,2152
Male n = 77 of 87 versus male n = 7 of 12	0,7946
With MSU n = 87 versus with Lipid liquid crystals n = 10 of 294	**0,4058**
Female n = 10 of 87 versus female n = 6 of 10	0,6855
Male n = 77 of 87 versus male n = 4 of 10	0,6855
With CPPD n = 180 versus with HA n = 4 of 294	**0,1136**
Female n = 101 of 180 versus female n = 2 of 4	0,2520
Male n = 79 of 180 versus male n = 2 of 4	0,4575
With CPPD n = 180 versus with Cholesterol n = 12 of 294	**0,0461**
Female n = 101 of 180 versus female n = 5 of 12	0,1848
Male n = 79 of 180 versus male n = 7 of 12	0,2723
With CPPD n = 180 versus with Lipid liquid crystals n = 10 of 294	**0,6572**
Female n = 101 of 180 versus female n = 6 of 10	0,6066
Male n = 79 of 180 versus male n = 4 of 10	0,9359
With HA n = 4 versus with Cholesterol n = 12 of 294	**0,0128**
Female n = 2 of 4 versus female n = 5 of 12	0,06514
Male n = 2 of 4 versus male n = 7 of 12	0,3454
With HA n = 4 versus with lipid liquid crystals n = 10 of 294	**0,0855**
Female n = 2 of 4 versus female n = 6 of 10	0,1445
Male n = 2 of 4 versus male n = 4 of 10	0,4284

Table 3: *Differences in mean age of 279 patients with various crystals of synovial fluids.*

Figure 1-8 demonstrate the morphology of MSU and CPPD crystals and the characteristic small prisms and clusters of HA crystals.

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.

The original magnification of electron microphotographs corresponds to the 60 x 90 mm negatives.

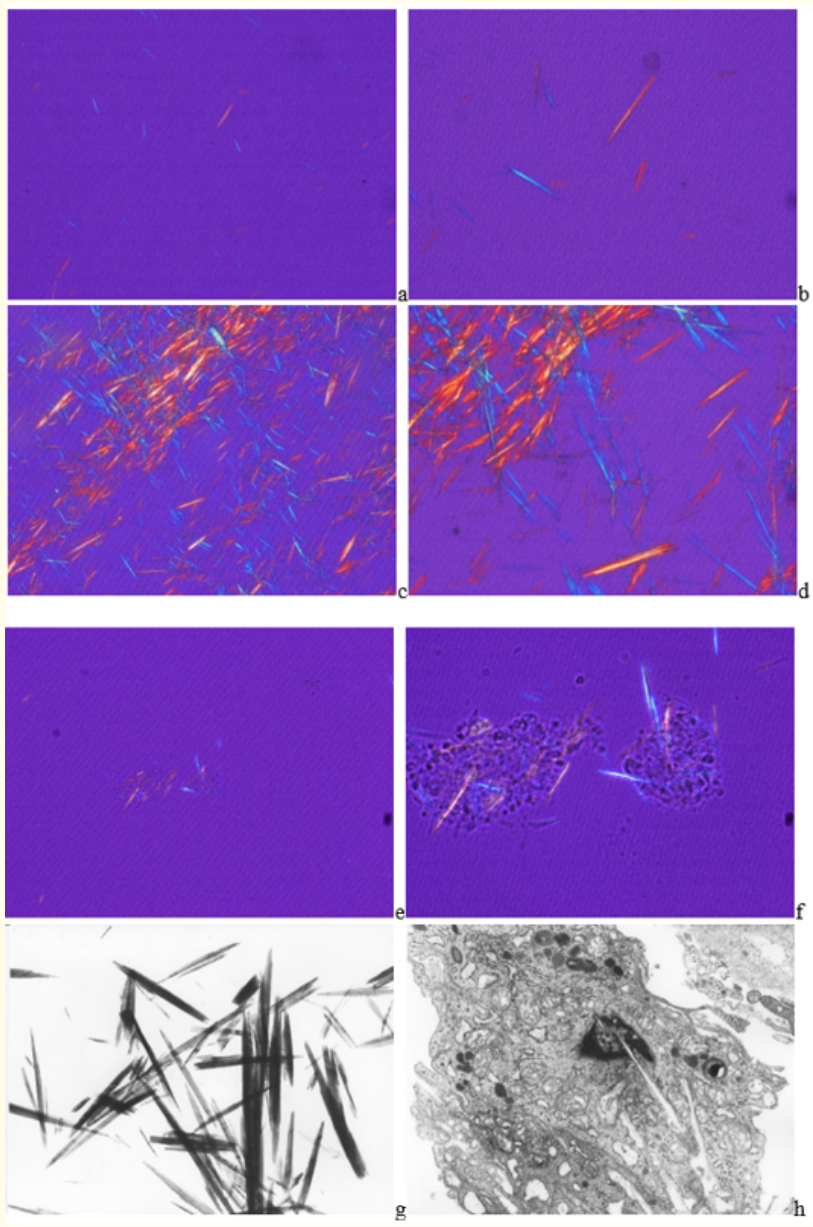

Figure 1a-1h: *(268/2005, 169/2005). Unstained wet preparation of synovial fluid.*

MSU [NaC5H3N4O3·H2O] crystals are needle-shaped spiked rods with very strong negative birefringence.

(a) Few MSU crystals, unstained wet preparation, viewed under polarized light, using Red I compensator, x200, (b) same as (a) x600, (c) Large amount of MSU crystals, unstained wet preparation, viewed under polarized light, using Red I compensator, x200, (d) same as (c) x600, (e) Phagocytosed MSU crystals, unstained wet preparation, viewed under polarized light, using Red I compensator, x200, (f) same as (e) x600, (g) MSU crystals, surface electron micrograph, x1600, (h) Phagocytosed MSU crystals, transmission electron micrograph, x6600.

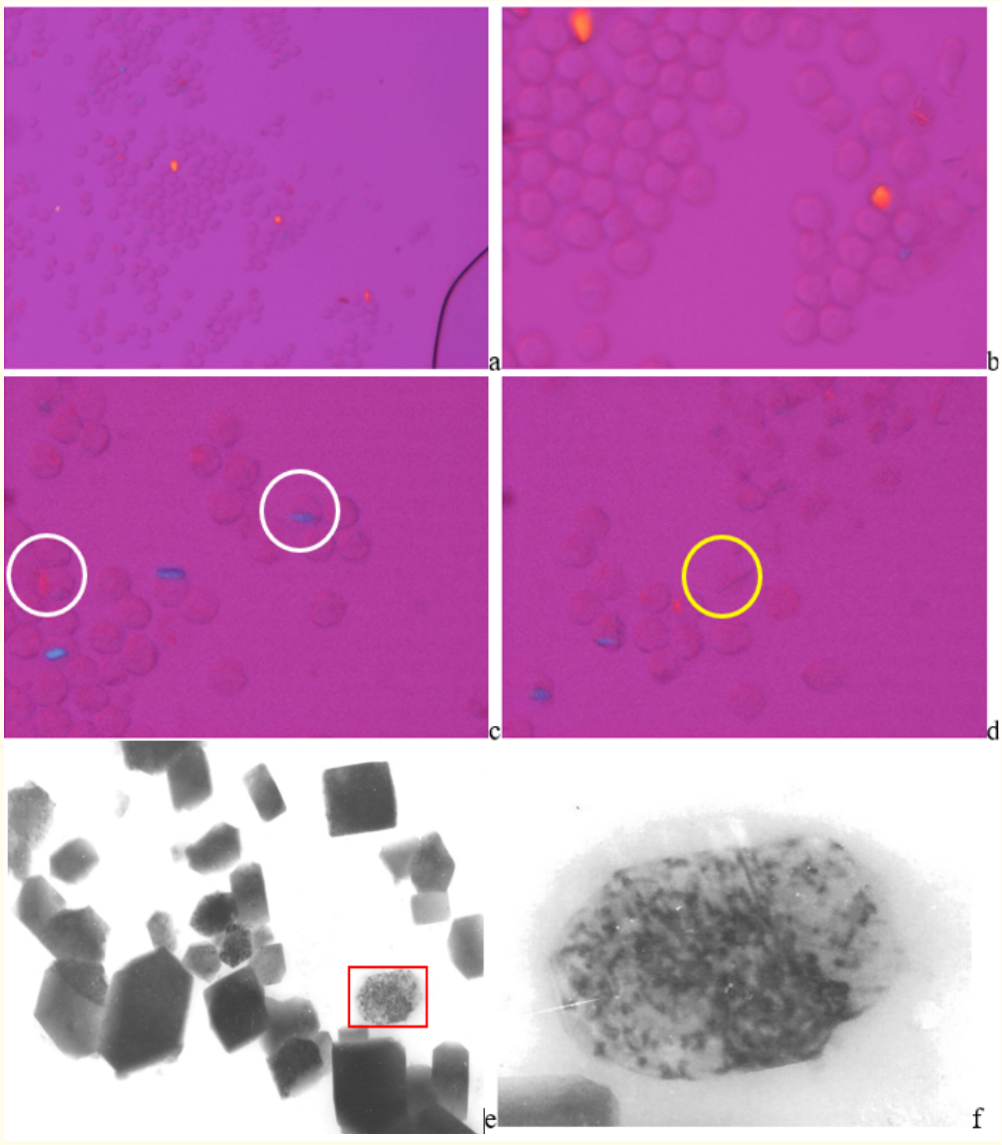

Figure 2a-2f: *(81/2020). Unstained synovial fluid wet preparation.*

CPPD [Ca2P2O7.2H2O] plane crystals are hexagonal, trapezoid rhomboid, parallelogram-shaped with positive birefringence.

(a) CPPD crystals, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator, x200, (b) same as (a) x600, (c) Phagocytosed CPPD crystals (white ellipses), unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator, x600, (d) phagocytosed crystal slice, probably cleaved cholesterol; the end of the narrow slice is blunt, not pointed (yellow ellipse), unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator, x600, (e) CPPD crystals, the shape of plane crystals is hexagonal. trapezoid rhomboid or parallelogram, surface electron micrograph, x10000, (f) Enlarged detail of (e), CPPD crystals and HA crystals, surface electron micrograph, x30000.

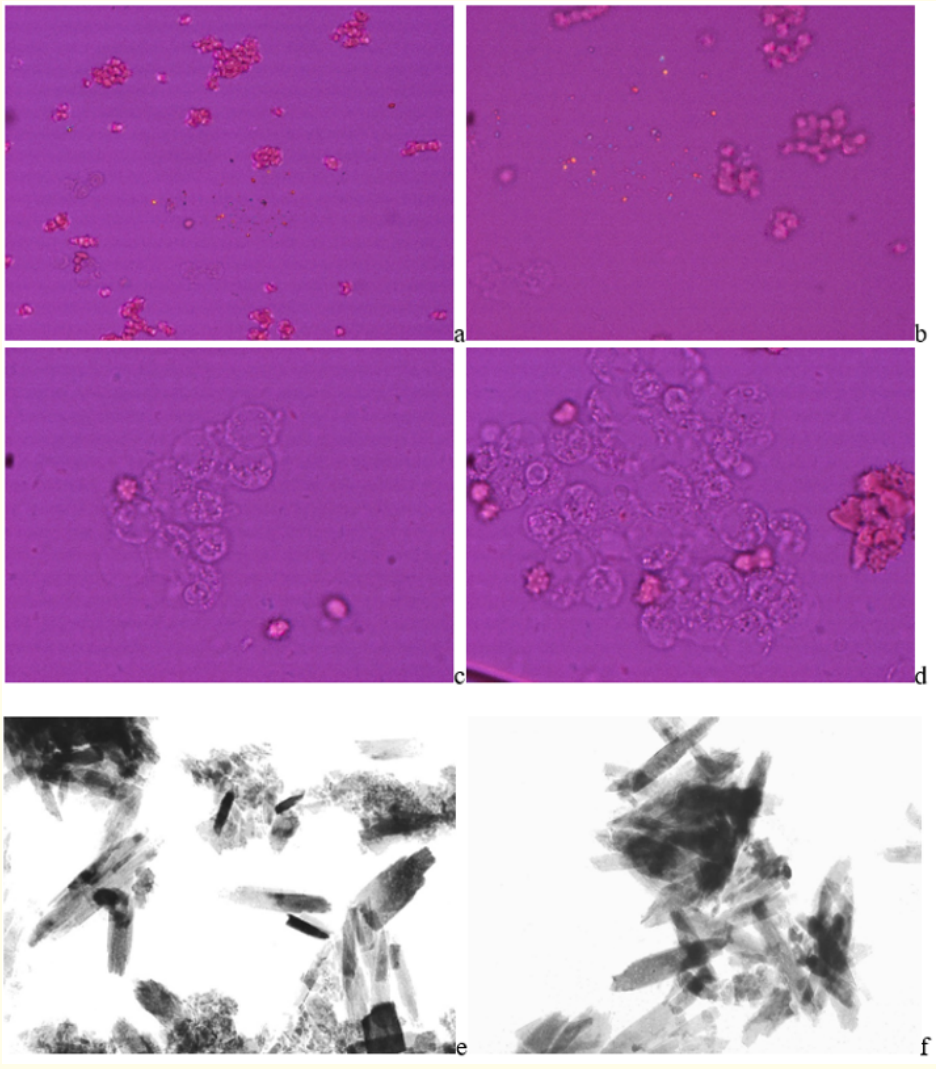

Figure 3a-3f: *(2170-2019). Right shoulder puncture, unstained synovial fluid wet preparation, first analysis of synovial fluid on 03.10.2019. Extracellular and phagocytosed intracellular small HA [Ca5(PO4)3(OH)] prisms; CPPD [Ca2P2O7.2H2O] crystals are not detected.*

Ten days after the first analysis of the same synovial fluid which was prepared freshly.

Most HA crystals are extracellular and are mixed with CPPD crystals (See figure 4a-4d).

This suggests the possible transformation of HA crystals into CPPD crystals, a possible transformation of HA crystals into CPPD crystals cannot be ruled out.

(a) Extracellular HA crystals, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator, x200, (b) same aspirate as (a), x400, (c) Intracellular phagocytosed HA crystals, unstained synovial fluid wet preparation, same aspirate as (a), x600, (d) Intracellular phagocytosed HA crystals, unstained synovial fluid wet preparation, same aspirate as (a), x600, (e) Cluster of rod-shaped HA crystals, surface electron micrograph, x50000, (f) Rod shaped HA crystals, surface electron micrograph, x50000.

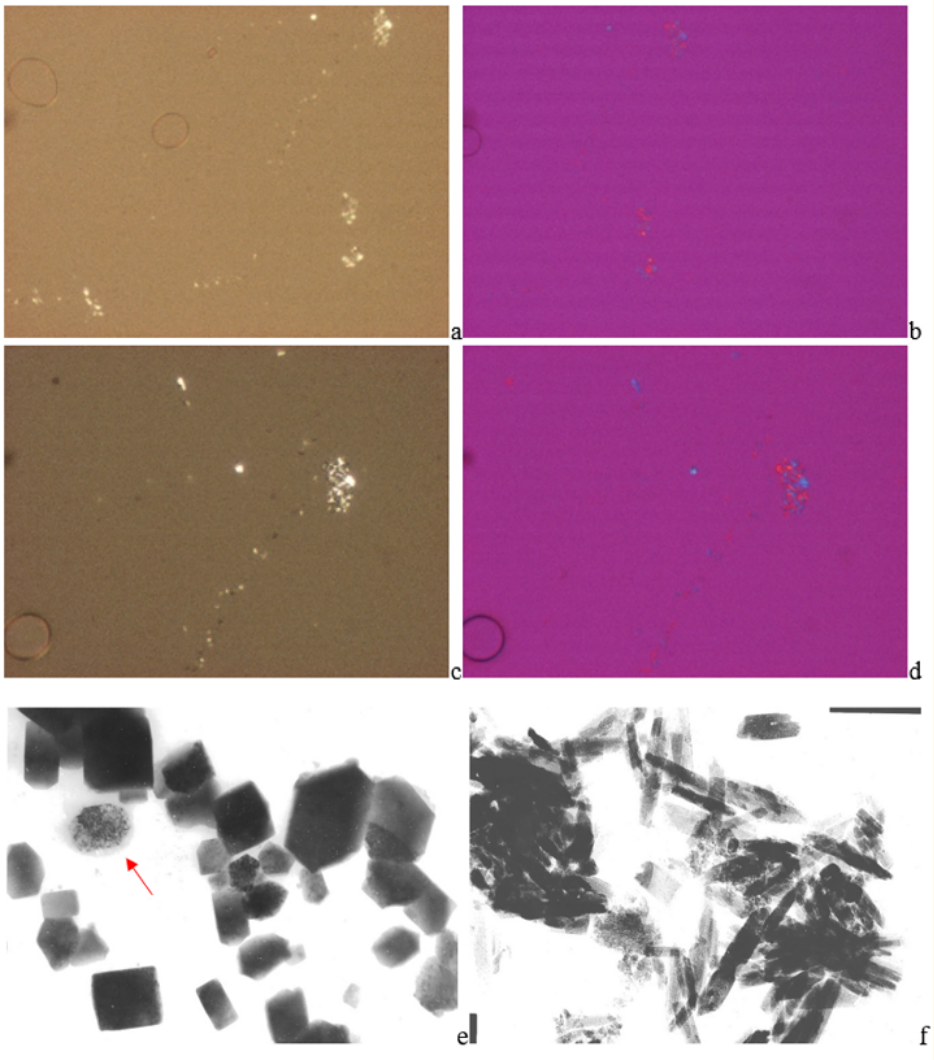

Figure 4a-4f: *(2170-2019). Right shoulder puncture, unstained synovial fluid wet preparation, first analysis of synovial fluid on 03.10.2019. Extracellular and phagocytosed intracellular small HA [Ca5(PO4)3(OH)] prisms; CPPD [Ca2P2O7.2H2O] crystals are not detected. Ten days after the first analysis of the same synovial fluid which was prepared freshly.*

Most HA crystals are extracellular and are mixed with CPPD crystals (See figure 4a-4d).

This suggests the possible transformation of HA crystals into CPPD crystals, a possible transformation of HA crystals into CPPD crystals cannot be ruled out.

(a) Extracellular HA crystals, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator, x200, (b) same aspirate as (a), x400, (c) Intracellular phagocytosed HA crystals, unstained synovial fluid wet preparation, same aspirate as (a), x600, (d) Intracellular phagocytosed HA crystals, unstained synovial fluid wet preparation, same aspirate as (a), x600, (e) Cluster of rod-shaped HA crystals, surface electron micrograph, x50000, (f) Rod shaped HA crystals, surface electron micrograph, x50000.

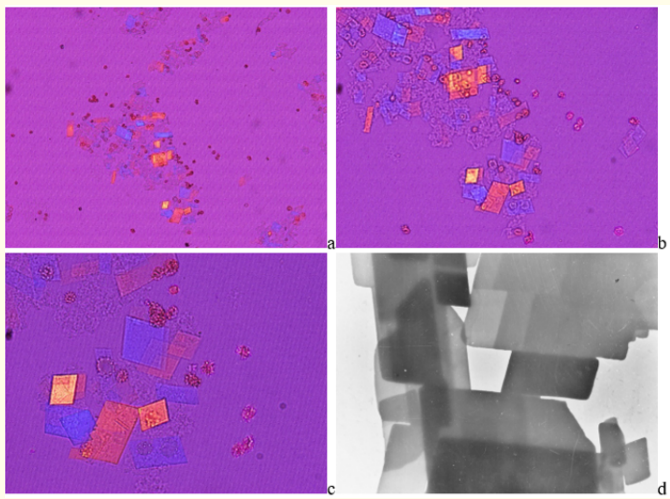

Figure 5a-5d: *(1119-2007). Unstained synovial fluid wet preparation, cholesterol [C27H46O] crystals.*

The cholesterol crystals are rhomboidal, notched, needle-shaped cleft and are present as separate sheets or typically arranged in clusters (see Figure 6a-d). A "semi-liquid" appearance is also characteristic (See figure 7a-7d).

The birefringence of cholesterol crystals is positive or negative; the needle-shaped or cleft crystal fragments rotating around the axis may show in the same position positive or negative birefringence.

(a) Extracellular cholesterol crystal plates, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) same as (a) x600, (d) Cholesterol crystals, surface electron micrograph, x1300.

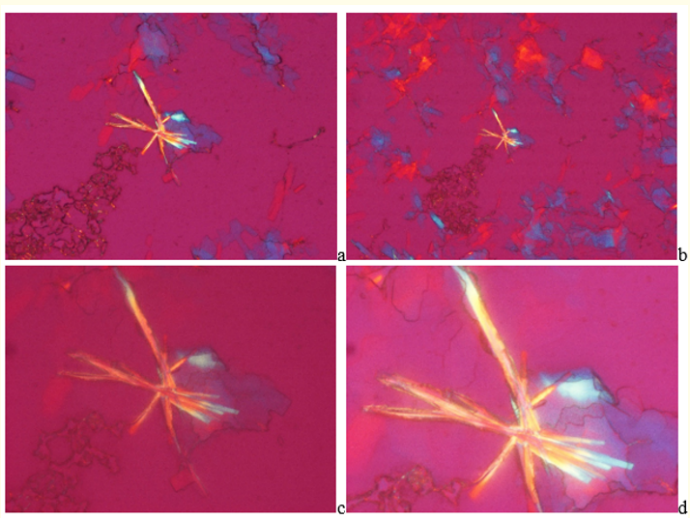

Figure 6a-6d: *(79-2002). Unstained synovial fluid wet preparation, cholesterol [C27H46O] crystals.*

The cleaved cholesterol crystals arranged in clusters (bundles).

The ends of the thin plates are blunt.

The birefringence of cholesterol crystals is positive or negative; the needle-shaped or cleft crystal fragments rotating around the axis may show in the same position positive or negative birefringence.

(a) Extracellular cholesterol crystal plates and clusters, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) same as (a) x400, (d) same as (a) x600.

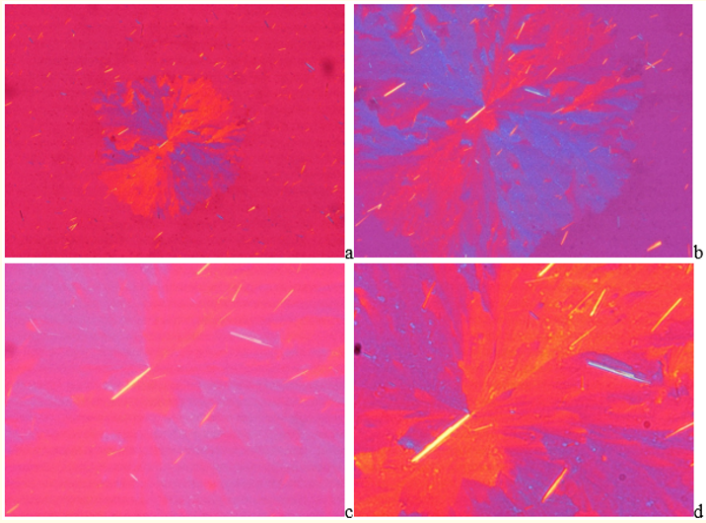

Figure 7a-7d: *(91-xx). Unstained synovial fluid wet preparation, cholesterol [C27H46O] crystals. Semiliquid cholesterol crystals with thin cleaved slices of cholesterol plates. The ends of the cleaved cholesterol slices are blunt, in contrast to the pointed ends of urate crystals (See figure 1a-1f). The birefringence of semiliquid crystals and cleaved cholesterol slices in this figure is negative. (a) Semiliquid cholesterol crystals with cleaved thin slices, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) same as (a) x400, (d) same as (a) x600.*

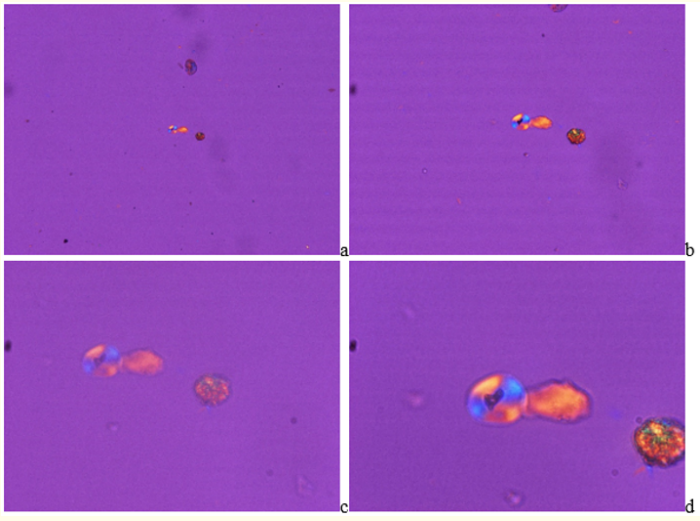

Figure 8a-8d: *(2510-2013). Unstained synovial fluid wet preparation, lipid liquid crystals with positive Maltese cross birefringence. (a) Extracellular lipid liquid crystals and phagocytosed intracellular lipids, unstained synovial fluid wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) same as (a) x400, (d) same as (a) x600.*

Discussion

It is generally accepted that crystalline particles may be associated with joint diseases, including MSU, CPPD, HA, cholesterol, lipid liquid crystals etc.

It is also well recognized that all of these crystals may be present in humans without manifest crystal induced metabolic disorders or arthropathies.

Hyperuricemia is essential for the formation of monosodium urate monohydrate MSU [$NaC_5H_3N_4O_3 \cdot H_2O$] crystals, but only a fraction of hyperuricemia patients develop gout (ranging from 2 to 36% of patients) [6].

In about two-thirds of people the elevated serum urate concentration causes neither symptoms nor signs of MSU crystal deposition disease, and remain asymptomatic, never developing gout flares, tophaceous gout, acute or chronic hypouricemic nephropathy, or uric acid nephrolithiasis [7].

The CPPD [$Ca_2P_2O_7.2H_2O$] crystal deposits may also exist without manifest or clinically diagnosed metabolic diseases [8]. Presence of CPPD crystals in the general population without complains is estimated between 4 to 7% of the adult population in Europe and in the United States [9].

The initial precalcific phase of apatite rheumatism due to HA [$Ca_5(PO_4)_3(OH)$] crystal deposits is also asymptomatic [10].

A crystal-induced metabolic disease was not evident, and crystal deposits of articular and periarticular tissues were not found in any of our patients with crystal positive synovial fluid (including MSU, CPPD, HA, etc. crystals).

The MSU crystals are needle-shapes spiked rods ranging in size from submicroscopic to 40 µm [11]. The very strong negative birefringence is characteristic to MSU crystals [11,17,18] (Figure 1a-1d).

CPPD is a typical plane crystal with positive birefringence. Different forms of CPPD may exist; hexagonal. trapezoid rhomboid, parallelogram-shaped or fragments of these (Figure 2a-2f). Their size range is 0.42 - 17.9 µm [10], according to some it varies from submicroscopic to 40 µm [11].

The individual HA crystals are small prisms, and may form clusters or aggregates of clusters.

Individual HA prisms are submicroscopic [19], according to others 50 - 500 nm [20], in clusters 1 - 5 µm [20] or 1.9 - 15.6 µm [11].

The HA crystals may associate sporadically with much larger and partially fragmented CPPD crystals (Figures 4a-4e).

The HA crystals stained with Alizarin red S "tend to aggregate …, but show no birefringence under compensated polarized microscopy (400 x)" [4].

Using a professional polarizing microscope with high, at least 100-Watt brightness the HA prisms are visible and birefringent with polarized light (100x) in non-stained synovial fluids [17,18].

The birefringence of HA prisms is weakly positive compared to the strong positive birefringence of CPPD crystals; the large and strongly birefringent CPPD crystals may deceptively dominate the microscopic fields in coexistent cases [17,18].

The size of cholesterol crystals is 5 - 40 µm [21], rhomboidal, notched, needle-shaped cleft and are present as separate sheets or typically arranged clusters.

The ends of the cleaved cholesterol slices, reminiscent of urate crystals, are blunt, in contrast to the pointed ends of urate crystals [22].

A "semi-liquid" appearance is also characteristic. The birefringence of cholesterol crystals is positive or negative; the needle-shaped or cleft crystal fragments rotating around the axis, and may show positive or negative birefringence in the same position [22].

The lipid crystals are small 0.5 - 30 μm spherules, with negative (Figure 9e) or positive (Figure 10d) Maltese cross birefringence [22].

Different crystals may exist simultaneously. Mituszova., *et al*. (1981) reported coexistent MSU and CPPD crystals in synovial fluid [23].

According to Reginato AJ and Reginato AM (2001) a rare combination of MSU and CPPD crystals may occur in synovial membranes or in synovial fluid [24].

Technical remarks

The examination of the synovial fluid should ideally be performed within 15 minutes of the puncture.

If this is not possible, the synovial fluid can be kept in a 4 C° ice box and the analysis of the freshly dropped fluid can be made at a convenient time; the result should be considered of limited value.

Examination of dried smears may show a colorful picture, but the dried liquid is unsuitable for meaningful examination (Figure 9-12).

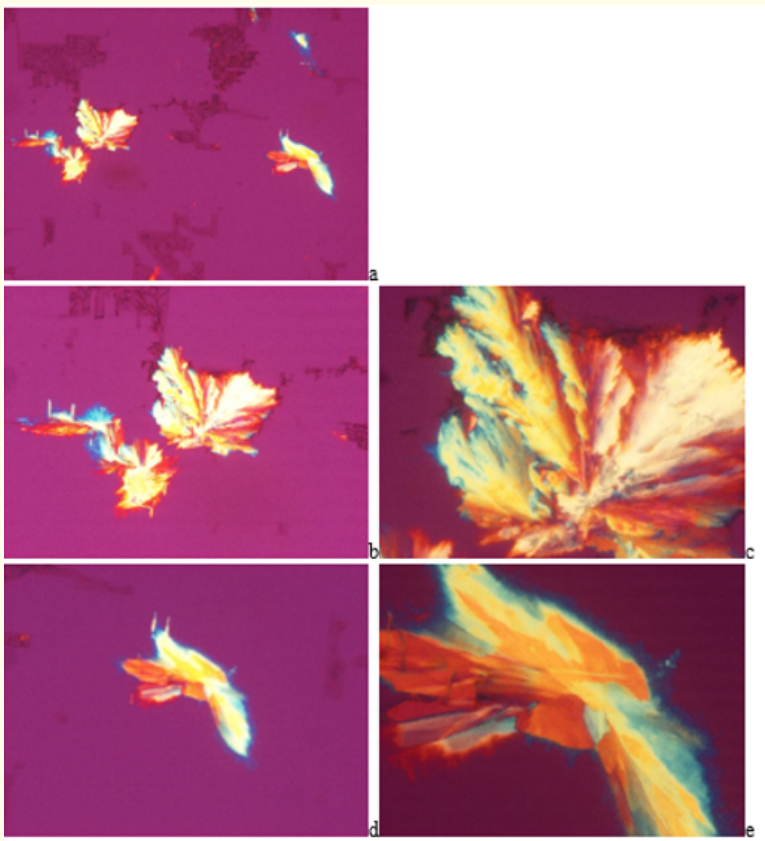

Figure 9a-9e: *(91-xx). Unstained synovial fluid, dried-up wet preparation, cholesterol [C27H46O] crystals.*
Bizarre shaped cholesterol crystals show positive and negative birefringence in the same direction.
(a) Bizarre shaped dried cholesterol crystals, unstained synovial fluid dried-up wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) same as (a) x200, (d) same as (a) x600, (e) same as (a) x600.

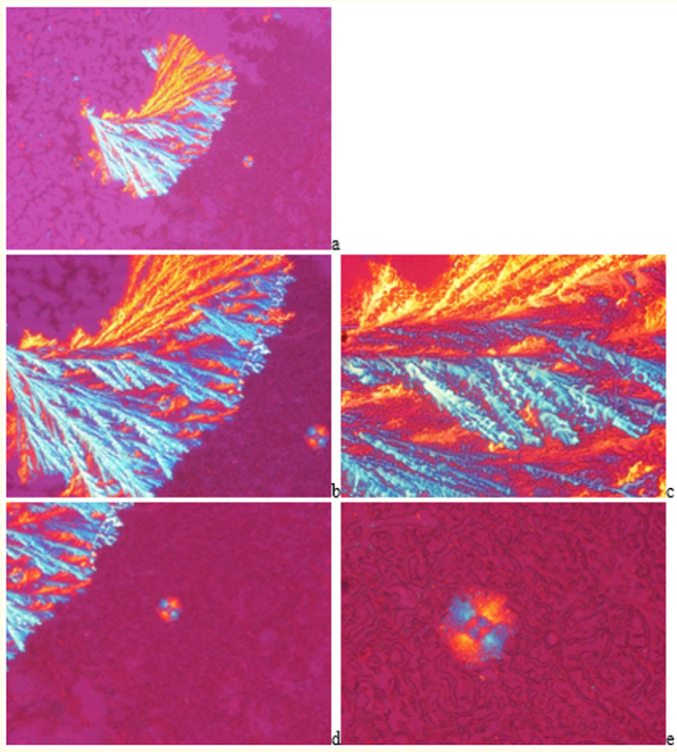

Figure 10a-10e: *(91-xx). Unstained synovial fluid, dried-up wet preparation, cholesterol [C27H46O] crystals.*
Bizarre shaped cholesterol crystals (reminiscent of snow crystals) and lipid liquid crystal with negative birefringence.
(a) Bizarre shape of dried-up cholesterol crystals, unstained synovial fluid dried-up wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) same as (a) x600
(d) Lipid liquid crystal, same field as (a) x200, (e) same as (d) x600.

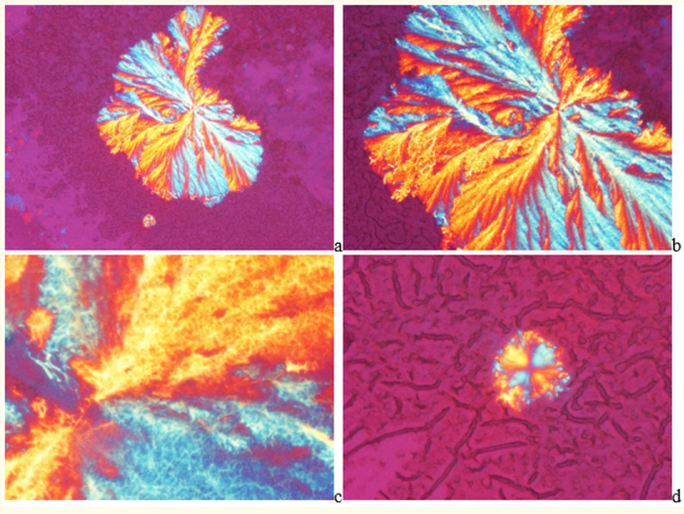

Figure 11a-11d: *(91-xx). Unstained synovial fluid, dried-up wet preparation, cholesterol [C27H46O] crystals.*
Bizarre shaped cholesterol crystals (reminiscent of snow crystals) with negative birefringence and lipid liquid crystal with positive birefringence.
(a) Bizarre shape of dried-up cholesterol crystals, unstained synovial fluid dried-up wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200, (c) x600, (d) lipid liquid crystal, same field as (a) x600.

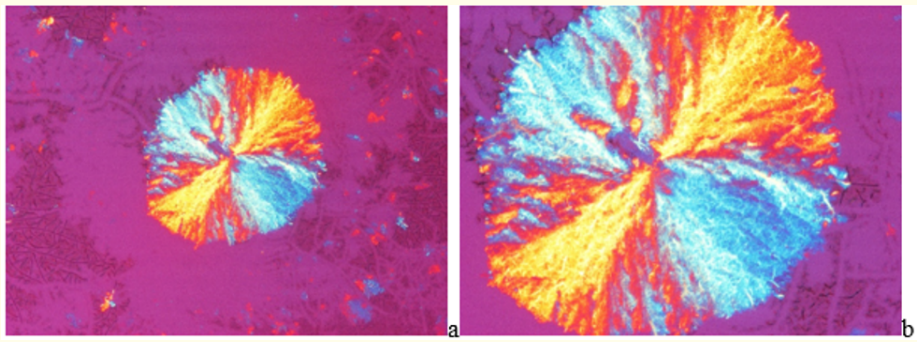

Figure 12a-12b: *(91-xx). Unstained synovial fluid, dried-up wet preparation, cholesterol [C27H46O] crystals. Bizarre shaped cholesterol crystals (reminiscent of snow crystals) with negative birefringence, and lipid liquid crystals with positive and negative birefringence.*
(a) Bizarre shape of dried-up cholesterol crystals, unstained synovial fluid dried-up wet preparation, viewed under polarized light, using Red I compensator x100, (b) same as (a) x200.

Staining of synovial fluids was exceptional in our cases; the dyes can resolve the crystals leading to a missed false negative diagnosis (especially in those cases where only a few crystals are present).

The probability of identification of crystals is much higher in unstained synovial fluid viewed under polarized light rather than in traditionally stained ones.

It is very important to use a professional polarizing microscope with high, at least 100-Watt brightness; the usual 60-Watt illumination may result in a false negative diagnosis.

Conclusion

Crystals may be present in the synovial fluid of patients without clinically diagnosed metabolic diseases or arthropathies.

The fast processing of synovial fluids, the exact technical circumstances, furthermore the use of a professional polarizing microscope with high, at least 100-Watt brightness are absolutely necessary for an accurate crystal analysis.

Acknowledgement

The authors are grateful to Rita Kisvölcsei for compiling the synovial aspiration list, and are gratefully acknowledging the help of cytology assistant Erika Jakabb in collecting the crystal positive findings).

Bibliography

1. Dieppe PA., *et al*. "Apatite associated destructive arthritis". *Rheumatology* 23.2 (1984): 84-91.

2. Garcia GM., *et al*. "Hydroxyapatite crystal deposition disease". *Seminars in Musculoskeletal Radiology* 7.3 (2003): 187-193.

3. Bachmann D and Resnick D. "Calcium pyrophosphate dihydrate crystal deposition disease" and "Calcium hydroxyapatite crystal deposition disease". In: Radiological atlas of rheumatological diseases, (Editors: Bachmann D, Resnick D), Editions Roche, F. Hoffmann-La Roche Ltd., Basel, Switzerland (1994): 108-116.

4. https://www.mayoclinic.org/diseases-conditions/pseudogout/symptoms-causes/syc-20376983

5. Reginato AM and Yuvienco C. "Hydroxyapatite Crystal-Induced". *Rheumatology* (2019).

6. Martillo MA., *et al*. "The Crystallization of Monosodium Urate". *Current Rheumatology Report* 16 (2014): 400.

7. Mount DB. "Asymptomatic hyperuricemia". Up To Date (2022).

8. Rosenthal AK and Ryan LM. "Calcium Pyrophosphate Deposition Disease". *The new England journal of Medicine* 374 (2016): 2575-2584.

9. Rosenthal AK. "Clinical manifestations and diagnosis of calcium pyrophosphate crystal deposition (CPPD) disease" (2022).

10. Uhthoff HK and Loehr JW. American Academy of Orthopaedic Surgeons 5.4 (1997): 183-191.

11. Gatter RA and Schumacher HR. "Microscopic findings under compensated polarized light and phase light". In: A practical handbook of joint synovial fluid analysis, 2nd edition. (Editors: Gatter RA, Schumacher HR), Lea and Febiger, Philadelphia, London (1991).

12. Carson FL. "Mayer's hematoxylin". In: Histotechnology (Editor: Carson FL), ASCP Press: Chicago (1990): 100-103.

13. McManus JFA and Mowry RW. "Methods of general utility for the routine study of tissues", "Sodium Alizarin sulfonate stain for calcium" and "Von Kossa's method for phosphates and carbonates" In: Staining methods, histologic and histochemical (Editors: McManus JFA, Mowry RW), Hoeber PB Inc, New York (1960): 55-72.

14. Vacca LL. "Alizarin red S" In: Laboratory manual of histochemistry (Editor: Vacca LL), Raven Press, New York (1985): 333-334.

15. Pearse AGE. "Fat soluble colorant methods" In: Histochemistry theoretical and applied, Volume two: analytical technology (Editor: Pearse AGE), Churchill Livingstone, Edinburgh, London, Melbourne and New York (1985): 799-807.

16. Lentner C. "Statistical methods" In Geigy scientific tables, 8th revised and enlarged ed. (Editor: Lentner C, compiled by: Diem K, Seldrup J), Ciba-Geigy Limited, Basle, Switzerland 2 (1982): 227.

17. Bély M and Apáthy A. "Metabolic Diseases and Crystal Induced Arthropathies Technic of Non-Staining Histologic Sections - A Comparative Study of Standard Stains and Histochemical Reactions". Clinical Archives of Bone and Joint Diseases 1 (2018): 2.

18. Bély M and Apáthy A. "Crystal deposits in tissue of patients with chondrocalcinosis and apatite rheumatism – Microscopic identification of CPPD and HA with the non-staining technique of Bely and Apáthy". *BAOJ Clinical Trials* 4 (2022): 018.

19. Swan A., *et al*. "Submicroscopic crystals in osteoarthritic synovial fluids". *Annals of the Rheumatic Diseases* 53.7 (1994): 467-470.

20. Pay S and Terkeltaub R. "Calcium pyrophosphate dihydrate and hydroxyapatite crystal deposition in the joint: New developments relevant to the clinician". *Current Rheumatology Reports* 5.3 (2003): 235-243.

21. Gardner DL and McClure J. "Metabolic, nutritional and endocrine diseases of connective tissue" In Pathological basis of the connective tissue diseases, 1st edition. (Editor: Gardner DL), Edward Arnold: London, Melbourne, Auckland, Great Britain 10 (1992): 380-393.

22. Bély M. "Szövettani vizsgálatok - A synovialis hártya (synovium) és extraarticularis lágyrészek kórjelező, diagnosztikus értékű elváltozásai autoimmun megbetegedésekben". 8. Fejezet, In: Reumatológia kézikönyve [Hungarian]. ("Histological examinations - Pathological, diagnostic changes of the synovial membrane (synovium) and extra-articular soft tissues in autoimmune diseases". In: Handbook of rheumatology) (Editors: Szekanecz Z and Nagy Gy), Medicina, Budapest, Hungary (2019): 99-121.

23. Mituszova M., *et al*. "Köszvény és rheumatoid arthritis együttes elôfordulása". *Orvosi Hetilap* 122 (1981): 339-342.

24. Reginato AJ and Reginato AM. "Gout and hyperuricemia" and "Diseases associated with deposition of calcium pyrophosphate or hydroxyapatite" In Crystal-associated synovitis, Section XV, Kelly's Textbook of Rheumatology, 6th ed. (Editors: Ruddy Sh, Harris ED jr, Sledge CB), WB Saunders Company: A division of Harcourt Brace and Company, Philadelphia, London, New York, St. Louis, Sydney, Toronto (2001): 1339-1376.

Volume 10 Issue 1 January 2023