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Case Report

Calcification and Crystal Deposition of Aortic Valves in Clinically Latent Metabolic Disease - A Case Report

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Abstract

A case of aortic valve disease due to atherosclerosis in combination with clinically latent metabolic disorders is presented using a novel microscopic technique.

Keywords: *Calcific Aortic Valve Disease; Atherosclerosis; Metabolic Disorders*

Abbreviations

AC: Arterial Calcification; CC: Cholesterol Crystals - [$C_{27}H_{46}O$]; CPPD: Calcium Pyrophosphate Dihydrate - [$Ca_2P_2O_7.2H_2O$]; HA: Calcium Hydroxyapatite - [$Ca_5(PO_4)_3(OH)$]; LDH: Lactic Dehydrogenase; MSU: Monosodium Urate - [$C_5H_4N_4O_3$]; H-E: Hematoxylin-Eosin

Introduction

Calcification of cardiac valves is a progressive disease akin to atherosclerosis with lipoprotein deposition, chronic inflammation, and active leaflet calcification [1]. However, there are differences between calcific valve disease and atherosclerosis which may be due to genetic polymorphisms, clinical risk factors (age, gender, body mass, hypercholesterolemia, elevated LDH or calcium level, chronic renal failure, diabetes mellitus, smoking etc.) [1-3].

Atherosclerosis is characterized by excessive deposits of cholesterol and cholesterol crystals (CC), accompanied by macrophages and an inflammatory cellular reaction contributing to the development and progression of atherosclerosis [4]. Hydroxyapatite (HA), calcium pyrophosphate dihydrate (CPPD), and monosodium urate (MSU) crystal deposition are linked to metabolic disorders independent of atherosclerosis [5-12].

This case demonstrates the metabolic diversities of deposits in calcific aortic valves.

Methods

Tissue blocks of surgically removed aortic leaflets were fixed in an 8% aqueous solution of formaldehyde at pH 7.6 for > 24 hours at room temperature (20°C), dehydrated and embedded in paraffin.

Serial sections (5 µm) were stained with H-E [13] and were compared with unstained sections according to Bély and Apáthy [14-16]. The fixation and embedding of tissue blocks was the standard one for staining and reactions, whereas unstained tissue sections

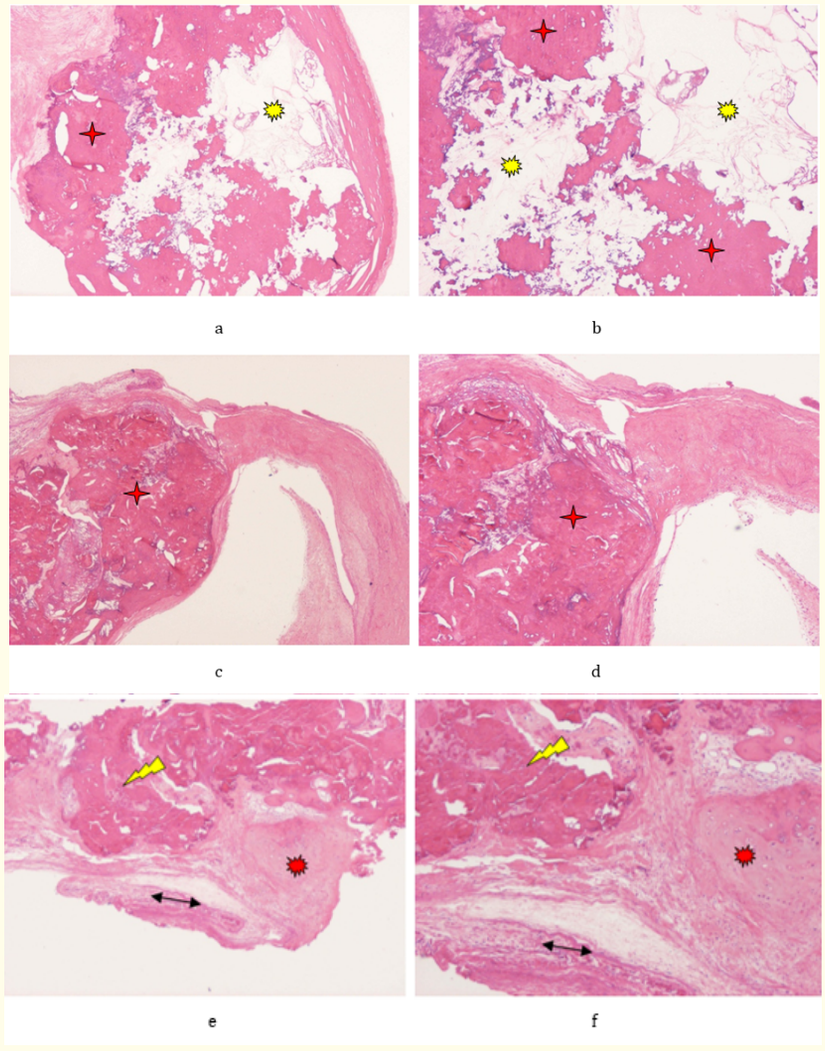
were deparaffinized, mounted and cover slipped with Canada balsam. This sensitive method is suitable to identify cholesterol (CC), hydroxyapatite (HA), calcium pyrophosphate dihydrate (CPPD), monosodium salt of uric acid (MSU) crystals etc. in formalin fixed, paraffin embedded, unstained tissue sections viewed with the light microscope (Olympus BX51) and under polarized light, respectively [14-17].

Case Report

This 85 year old man had known aortic stenosis for many years. He also had a single attack of gout (right great toe) 20 years ago and has daily been taking allopurinol (200 mg) without any further symptoms. Lab tests revealed hyperlipidemia. Over the last two months he noted chest pain with exertion, dyspnoe on exertion and palpitations. A stress echocardiogram revealed severe aortic stenosis for which he underwent aortic valve replacement with a 25 mm St. Jude tissue valve. Three intact semilunar valve cusps were submitted for microscopic examination.

Surgical pathology report: Calcific degeneration with amorphous calcific debris, focal chondroid and osseous metaplasia with lamellar bone formation accompanied by deposits of CC, CPPD, HA crystals and occasional MSU crystals in cardiac valves.

Figure 1-6 demonstrate the calcification of aortic valves in combination with crystal deposition of metabolic disorders. The results of traditional staining and reactions are compared with polarized light observations of the non-staining technique.



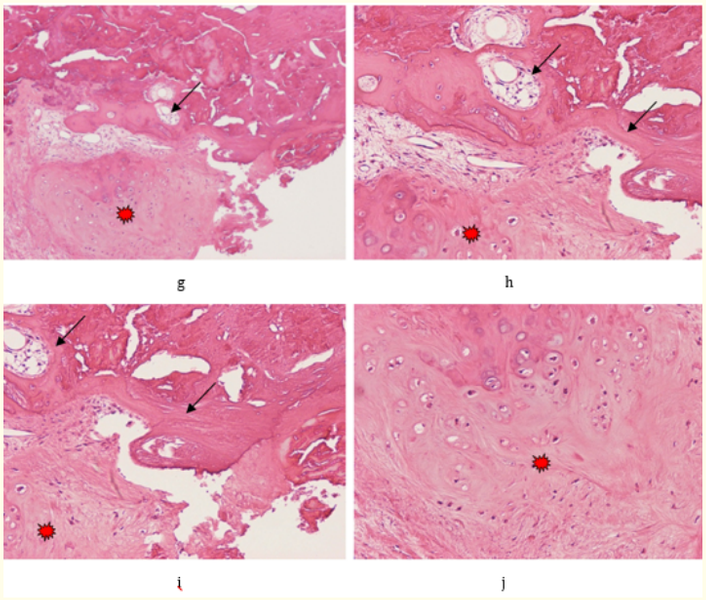


Figure 1a-1j: *Aortic valves, atherosclerosis with lipid deposits, amorphous calcification, chondroid metaplasia and bone formation (stained with H-E).*

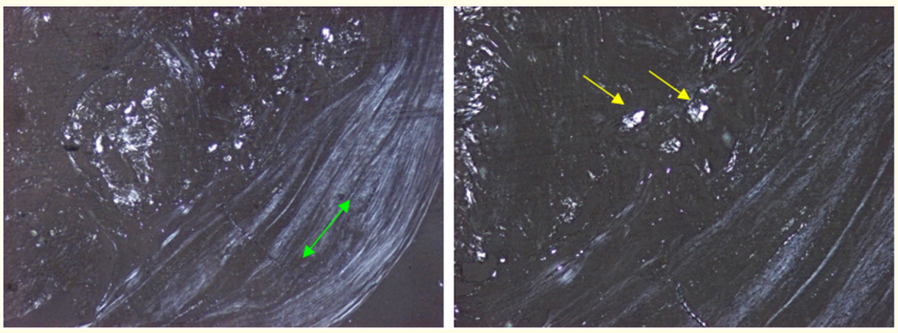
(a-b) Lipid deposits with foam cells (yellow star), and amorphous calcification (red star) within aortic valve (a) H-E, x 20, (b) same as (a) x40.

(c-d) Massive amorphous calcification (red star), within aortic valve, (c) H-E, x 20, (d) same as (c) x40.

(e-f) Subendothelial lipid deposition (arrow heads), woven bone formation (yellow arrow), and chondroid metaplasia (red star), (e) H-E, x 20, (f) same as (e) x40.

(g-h) Lamellar bone formation with true medulary spaces (black arrows), and chondroid metaplasia (red star), (g) H-E, x 40, (h) same as (g) x100.

(i-j) Lamellar bone formation with true medulary spaces, and chondroid metaplasia (red star), (i) H-E, x 100, (j) same as (i) x100.



a ***b***

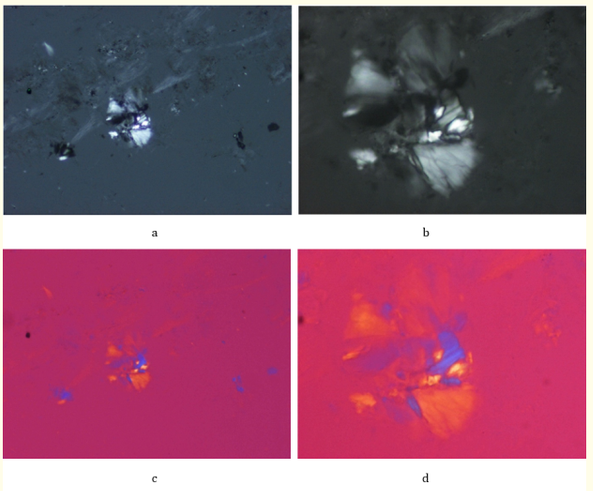
Figure 2a and 2b: *(Same as Figure 1, unstained section).*

Subendothelial fibrosis of aortic valve with cholesterol, HA, and CPPD crystal deposits.

Collagen fibers (marked with green arrowheads), fragments of cholesterol crystals (yellow arrows) combined with HA and CPPD deposits in coincidental metabolic disorder.

In traditionally fixed, H-E stained sections the cholesterol and HA crystals (crystal clusters and aggregates of clusters) dissolve and thus are not demonstrable. CPPD crystals may be preserved, but the few sporadic CPPD crystals or fragments are usually not visible.

(a) Fragments of cholesterol and CPPD crystals (with strong birefringence) and clusters of needle shaped, rod like HA crystals (with weak birefringence), non-stained section viewed under polarized light, x40, (b) same as (a) x100.



3a-3d: *(Unstained section).*

Fibrotic aortic valve with cholesterol crystals.

(a) Needle shaped cholesterol crystals typically arranged in clusters, viewed under polarized light x 400, (b) same as (a) x600.

(c) The birefringence of cholesterol crystals is positive or negative; the needle-shaped crystal fragments rotating around the axis may show in the same position (direction) positive or negative birefringence (in this Figure the breaking direction is positive). Unstained section, Red I compensator, viewed under polarized light, same as (a) x400, (d) same as (b) x600.

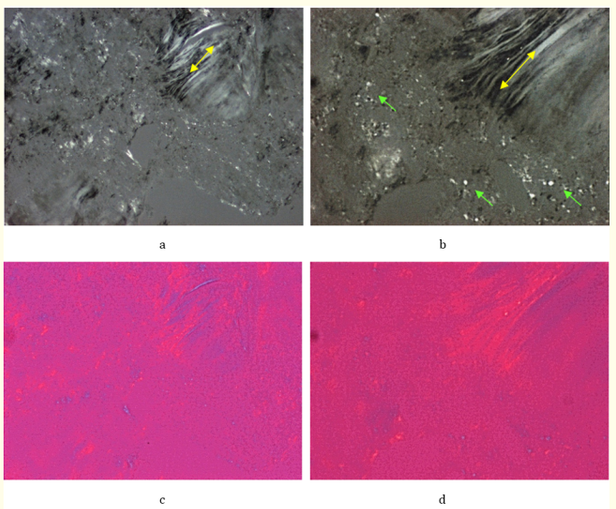


Figure 4a and 4d: *(Same patient as figure 1, unstained section).*

Ossification of aortic valve with crystal deposits.

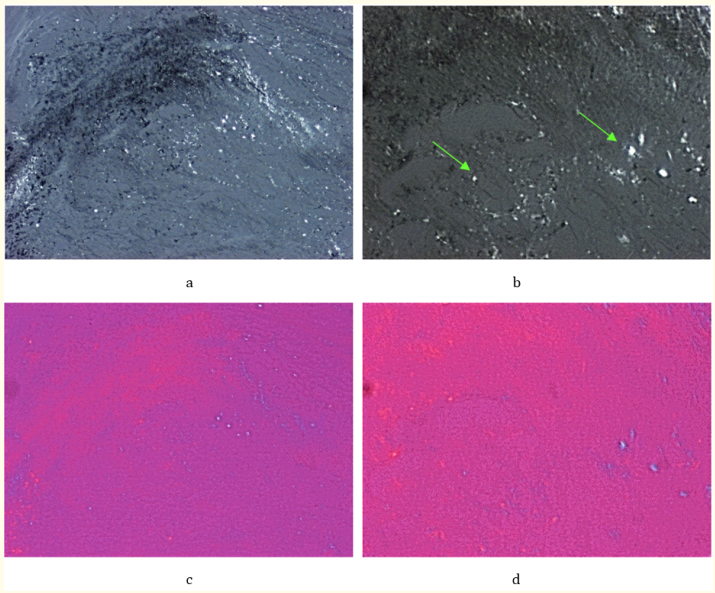
Woven bone (birefringence is stronger than that of collagen fibers in fibrous part of aortic valves; marked with yellow arrowheads), partially fragmented CPPD crystals (green arrows) combined with HA deposits in the coincidental metabolic disorder.

The individual HA crystals are small, 50-500 nm, rod-shaped and are arranged typically in 1-5 µm spheroid microaggregates which are not in visible range with polarizing microscopy. The figure a and b demonstrate crystal clusters and aggregates of clusters of 6.5 and 20 µm size, which may appear under polarized light using a professional polarizing microscope with high brightness, the clusters show week birefringence. Under polarized light the birefringence is positive according to the long axis of HA crystals, like that of collagen fibers.

The HA crystal clusters (microaggregates) and aggregates of clusters are associated sporadically with much larger and partially fragmented CPPD crystals. The CPPD crystals are less soluble than HA crystals or crystal aggregates. The birefringence of CPPD crystals is stronger than that of HA crystals or crystal aggregates.

(a) CPPD (with strong birefringence) and clusters of needle shaped, rod like HA crystals (with weak birefringence), Bély and Apáthy's non-staining technique, viewed under polarized light, x200, (b) same as (a) x600.

(c) Unstained section, Red I compensator, viewed under polarized light, same field as (a) x200, (d) same as (b) x600 Axis parallel direction of birefringence of HA and CPPD crystals are positive (identical with collagen fibers).

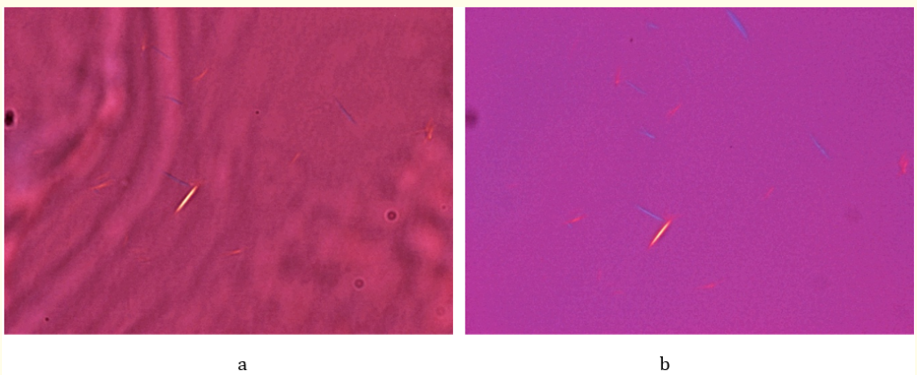


5a-5d: *(Unstained section).*

Loose part of aortic valve with HA and CPPD crystal deposits.

(a) Partially fragmented CPPD crystals (with strong birefringence; marked with green arrows) and clusters of needle shaped, rod like HA crystals and aggregates of clusters (with weak birefringence), Bély and Apáthy's non-staining technique, viewed under polarized light, x200, (b) same as (a) x600.

(c) Unstained section, Red I compensator, viewed under polarized light, same field as (a) x200, (d) same as (b) x600, axis parallel direction of birefringence of HA and CPPD crystals are positive (identical with collagen fibers).



6a-6b: *Atherosclerotic aortic valve combined with metabolic disorder and clinically latent gout (unstained section).*

The individual MSU crystals are needle-shaped, < 4 µm, and are typically arranged in bundles. MSU crystals are not present in H-E stained sections (except if in extremely large amounts) of 8% formaldehyde fixed specimens.

MSU crystals are present in unstained sections of 8% formaldehyde fixed specimens, showing a strong negative axis parallel birefringence with Rot I. compensator.

(a) Unstained section, Red I compensator, viewed under polarized light, x 600, (b) same as (a) x600.

Original magnifications of all figures correspond to the 24 x 36 mm transparency slide; the correct height: width ratio is 2:3. The printed size may be different, therefore the original magnifications are indicated.

Discussion

Coincidence of different diseases in one patient may occur, atherosclerosis may be combined with metabolic disorders as well, and the classic atherosclerotic calcification (with empty spaces of dissolved cholesterol crystals, ossification, etc. [18-21], may be associated with deposition of various other crystals [22-24,30].

Figure 7 demonstrates the classic aortic valve disease of atherosclerosis with traditional staining and reaction.

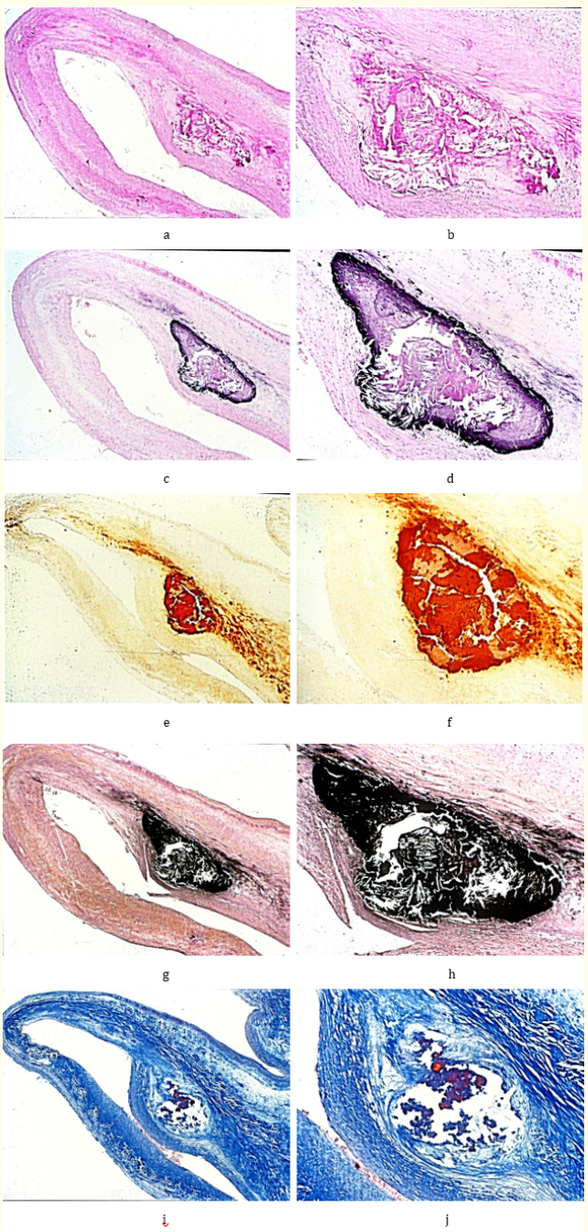


Figure 7a-7j: *Aortic valves, atherosclerosis with lipid deposits, amorphous calcification and chondroid metaplasia*
(a-b) Lipid deposits with characteristic spaces of dissolved cholesterol crystals stained with H-E [13], (a) H-E x 20, (b) same as (a) x50.
(c-d) Amorphous calcification of blue-violet colour with H-E, (c) H-E, same as (a) x 20, (d) same as (c) x50.
(e-f) Massive, amorphous calcium containing mineral deposits are staining with calcium specific Alizarin red S [25-26], (e) Alizarin Red S staining, viewed with the light microscope, same as (a) x20, (f) same as (e) x50.
(g-h) Amorphous phosphate or carbonate containing mineral deposits show positive reaction according to von Kossa [25-27], (g) von Kossa's reaction, viewed with the light microscope, same as (a) x20, (h) same as (g) x50.
(i-j) Hyaline substances and fibrin stain red with Azan [28], (i) Azan, same as (a) x20, (b) same as (i) x50.

Coexisting complications modify the basic pathological processes of calcific valve syndrome due to atherosclerosis and may cause differences in clinical manifestation or progression of symptoms. Coincidental clinically latent or manifest metabolic disorders contribute to aortic valve stenosis.

Conclusion

The patient's history of aortic valve stenosis was in combination with clinically latent metabolic disorders, characterized by different crystal deposition.

With Bély and Apáthy's "non-staining" technique cholesterol, hydroxyapatite (HA), calcium pyrophosphate dihydrate (CPPD), monosodium urate (MSU) crystals were identified in formalin fixed, paraffin embedded tissue sections.

Bibliography

1. Freeman RV and Otto CM. "Spectrum of calcific aortic valve disease: pathogenesis, disease progression, and treatment strategies". *Circulatio*n 111.24 (2005): 3316-3326.

2. Nasir K., *et al.* "Ethnic differences between extra-coronary measures on cardiac computed tomography: multi-ethnic study of atherosclerosis (MESA)". *Atherosclerosis* 198.1 (2008): 104-114.

3. Cowell SJ., *et al.* "Calcific aortic stenosis: same old story?" *Age Ageing* 33.6 (2004): 538-544.

4. Grebe A and Latz E. "Cholesterol crystals and inflammation". *Current Rheumatology Reports* 15.3 (2013): 313.

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

6. Dieppe PA., *et al.* "Apatite deposition disease. A new arthropathy". *Lancet* 307 (1976): 266-269.

7. Halverson PB., *et al.* "Histopathological and ultrastructural studies of synovium in Milwaukee shoulder syndrome - A basic calcium phosphate crystal arthropathy". *Annals of the Rheumatic Diseases* 43.5 (1984): 734-741.

8. Gardner DL and McClure J. "Metabolic nutritional and endocrine diseases of connective tissue, Crystal deposition disease, Urate deposition disease (gout), Calcium pyrophosphate dihydrate crystal deposition disease (chondrocalcinosis; pseudogout), Calcifying tenosynovits, Calcium hydroxyapatite and articular disease". In Pathological Basis of the Connective Tissue Diseases, 1st edition: Edward Arnold, London, Melburne, Auckland, Great Britain, Editor: Gardner DL 10 (1992): 380-393.

9. Mohr W. "Gicht, Kalziumpirophosphat-Arthropathie, Apatitkrankheiten". In Gelenkpathologie, historische Grundlagen, Ursachen und Entwicklungen von Gelenkleiden und ihre Pathomorphologie. Berlin, Heidelberg, Springer-Verlag, Germany, Editor (2000): 193-201.

10. Reginato AJ and Reginato AM. "Diseases associated with deposition of calcium pyrophosphate or hydroxyapatite". In Crystal-associated Synovitis, Section XV, Kelly's Textbook of Rheumatology, 6th edition. WB Saunders Company: A Division of Harcourt Brace & Company, Philadelphia, London, New York, St. Louis, Sydney, Toronto, Editors: Ruddy Sh, Harris ED Jr, Sledge CB (2001): 1377-1390.

11. Fassbender HG. "Crystal-associated arthropathies, Gout, Calcium pyrophosphate dihydrate crystal deposition disease, Hydroxyapatite crystal deposition disease". In Pathology and Pathobiology of Rheumatic Diseases, 2nd ed. Berlin, Heidelberg, New York, Springer-Verlag, Germany, Editor: Fassbender HG 17 (2002): 353-369.

12. Gupta SJ. "Crystal induced arthritis: an overview". *INJR (Indian Journal of Rheumatology - formerly: Journal of Indian Rheumatology Association* 10 (2002): 5-13.

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

14. Bély M and Apáthy Á. "A Simple Method of Diagnostic Pathology for Identification of Crystal Deposits in Metabolic and Crystal Induced Diseases". *Structural Chemistry and Crystallography Communication* 2.1-15 (2016): 1-6.

15. 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.

16. 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: 018.

17. Bély M and Krutsay M. "A simple method to demonstrate urate crystals in formalin fixed tissue". *Journal of Autoimmune Diseases and Rheumatology (JADR)* 1 (2013): 46-49.

18. Baumer Y., *et al.* "Hyperlipidemia-induced cholesterol crystal production by endothelial cells promotes atherogenesis". *Nature Communications* 8 (2017): 1129.

19. Grebe A and Latz E. "Cholesterol crystals and inflammation". *Current Rheumatology Reports* 15.3 (2013): 313.

20. Deneke T., *et al.* "Ossification in atherosclerotic carotid arteries". *Zeitschrift für Kardiologie* 90.3 (2001): 106-115.

21. Hunt JL., *et al.* "Bone Formation in Carotid Plaques A Clinicopathological Study". *Stroke* 33 (2002): 1214-1219.

22. Bély M and Apáthy A. "Mönckeberg-sclerosis - kristály indukálta angiopathia. (Mönckeberg's sclerosis - crystal induced angiopathy)". *Orvosi Hetilap* 154.23 (2013): 908-913.

23. Bély M and Apáthy A. "Functional Role of Hydroxyapatite Crystals in Monckeberg's Arteriosclerosis". *Journal of Cardiovascular Disease* 2.5 (2014): 228-234.

24. Quaglino D., *et al.* "The biology of vascular calcification". *International Review of Cell and Molecular Biology* 354 (2020): 261-353.

25. 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, Hoeber PB Inc, New York, Editors: McManus JFA, Mowry RW 55-72 (1960): 193-194.

26. Vacca LL. "Alizarin red S". In Laboratory Manual of Histochemistry, Raven Press, New York, Editor: Vacca LL (1985): 333-334.

27. Lillie RD. "Von Kóssa's method In Histpathologic technic and practical histochemistry". The Blakiston Division McGraw-Hill Book Company, New York, Toronto, London, Editor: Lillie RD (1954): 264-265.

28. Endes P. "Kombinált trichróm festés (Főleg kötőszövet és az érfalak degeneratív elváltozásainak feltüntetésére). (Combined trichrom staining; chiefly with reference to degenerative changes of the connective tissuee and arterial walls.)". *Kísérletes Orvostudomány* 6.5 (1954): 479-480.

29. Aortic valve stenosis by By Mayo Clinic Staff.

30. Gawoski JM., *et al.* "Aortic valvular tophus: Identification by X-ray diffraction of urate and calcium phosphates". *Journal of Clinical Pathology* 38 (1985): 873-876.

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