Histol Histopathol (2008) 23: 137-141

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Histochemical demonstration of aluminum and iron deposition in pulmonary bony tissues in three cases of diffuse pulmonary ossification

Yuji Ohtsuki[1], Akira Yamanaka[2], Hideki Ohyama[3], Eiji Yamada[4], Nobuyuki Terada[3], Jiro Fujita[5], Gang-Hong Lee[6] and Mutsuo Furihata[6]

[1]Division of Pathology, Matsuyama-shimin Hospital, Matsuyama, Ehime, Japan, [2]Department of Respiratory Diseases and Chest Surgery, Otsu Red Cross Hospital, Otsu, Japan, [3]Department of Pathology, Hyogo College of Medicine, Nishinomiya, Japan, [4]Laboratory of Diagnostic Pathology, Hikone Municipal Hospital, Hikone, Japan, [5]Department of Medicine and Therapeutics, Control and Prevention of Infectious Diseases, Faculty of Medicine, University of the Ryukyu, Okinawa, Japan and [6]Department of Pathology, Kochi Medical School, Kochi University, Kochi, Japan

Summary. Diffuse pulmonary ossification is a rare condition. We examined three cases of it in Japan, and attempted histochemically to stain for deposition of aluminum and iron in bony tissues. The patients were all female, and in their mid-twenties, mid- eighties, and later teen years. One of the patients had been exposed to heavy metals in her work involving heavy-metal analyses for 18 months. Aluminum staining and Berlin blue staining for iron were performed with dewaxed, undecalcified sections of pulmonary tissues from these three cases.

Interestingly, all pulmonary bony tissues from the three cases examined exhibited linear regions of both aluminum and iron deposition in the calcifying fronts or the cement lines of bones. The patient exposed to heavy metals exhibited the most severe aluminum and iron deposition, and also exhibited positive reaction for both aluminum and iron in elastic fibers of blood vessels. Foreign body granulomas with multinucleated giant cells exhibiting elastophagia were also found in this case. This phenomenon, "endogenous pneumoconiosis", appeared to have been the cause of pulmonary hemorrhage in this case, resulting in focal heavy hemosiderosis. It is of great interest that identical patterns of aluminum and iron deposition in hemodialysis patients were found in these three cases,

This is the first report on histochemical demonstration of aluminum and iron deposition in diffuse pulmonary ossification, and detailed analysis of additional cases is needed.

Offprint requests to: Yuji Ohtsuki, MD, PhD, Division of Pathology, Matsuyama-shimin Hospital, Matsuyama Ehime 790-0067, Japan. e-mail: y.ohtsuki@matsuyama-shimin-hsp.or.jp

Key words: Diffuse pulmonary ossification, Aluminium, Iron, Histochemistry, Endogenous pneumoconiosis

Introduction

Diffuse pulmonary ossification (DPO) is a rare condition, and is divided into two types, dendriform and nodular (Tseung and Duflou, 2006). DPO is usually diagnosed at autopsy, and not clinically.

Aluminum and/or iron deposition has generally been tested for in the bony tissues of hemodialysis patients with renal osteodystrophy by histochemical staining (Pierides and Myli, 1984; Van de Vyver et al., 1984; Verbueken et al., 1984, Ohtsuki et al., 1989, 1992), X-ray microprobe analysis (Van de Vyver et al., 1984, Pai et al., 1994), ultrastructural analysis (Verbueken et al., 1984; Pai et al., 1994), and other methods. For aluminum staining, in particular, we have devised a simple method using dewaxed sections, similar to that previously reported for iron staining (Ohtsuki et al., 1989), and used it instead of the methyl methacrylate embedding method (Maloney et al., 1982).

In the present study, we attempted to stain the iron and aluminum in pulmonary bony tissues histochemically, using dewaxed sections, since it was noted that the calcifying fronts of these bones were dense black or deep purple in color on hematoxylin-eosin staining.

Materials and methods

Materials from three female subjects, one in her mid-twenties (case 1), one in her mid-eighties (case 2), and one in her late teens (case 3), were examined.

In case 1, exposure to heavy metals occurred for 18 months due to work in heavy-metal analyses. An abnormality was found on chest X-ray, and video-assisted thoracoscopic surgery (VATS) was subsequently performed for precise histopathological diagnosis. Clinically, interstitial pneumonia was suspected. Histopathologic examination revealed DPO, dendriform type, with focal heavy hemorrhage, and fatty marrow in some bony tissues. Two pulmonary sections containing bony tissue embedded in paraffin were examined.

For case 2, clinicopathological findings were reported elsewhere in detail (Ohyama et al., 2006). In brief, she was treated for ten years due to diabetes mellitus and hypertension. One month before her admission she complained of chest pain and dyspnea, and then, left ventricular hypertrophy and atrial fibrillation due to tachycardia were pointed out. Then, her clinical course was getting worse despite intensive therapy, and died of cardiac and renal insufficiency, and sepsis one month later. At autopsy, DPO, dendriform type was incidentally found in all pulmonary lobes, associating cardiac hypertrophy with infectious verrucous endocarditis of mitral valve as the cause of sepsis. Marked hyalinization of islets in pancreas and diabetic nephropathy were found. Two representative pulmonary sections containing bony tissues were examined.

In case 3, adult-type Still's disease had been diagnosed clinically. During treatment, she complained of sudden development of gastrointestinal symptoms, such as vomiting, diarrhea, and nausea, and died of cardiac and pulmonary function arrest. DPO of dendriform type was incidentally detected in the lungs at autopsy. One representative pulmonary section containing bony tissues was examined.

A detailed description of histochemical staining for aluminum was reported elsewhere (Ohtsuki et al., 1989). In the present study, no decalcification processes of pulmonary tissues were done, and each dewaxed section in all three cases were used. Iron staining was performed using standard Berlin blue stain.

Results

All three cases involved the dendriform type of DPO, and some bony tissues exhibited fatty marrow without hematopoiesis.

The bony tissues were round, irregularly nodular, curved, or branching in form, and dense black lines or fine black granules were clearly observed in calcifying fronts or cement lines even on HE staining (Fig. 1a), especially in cases 1 and 2. On aluminum staining, linear positive reaction was found in most of the calcifying fronts of bony tissues (Fig. 1b), and some cement lines were also positive as well on iron staining (Fig. 1c). While most were linear, some diffuse regions of positivity for aluminum in bony tissues were detected in case 2 (Fig. 2a). Osteoid tissues were always negative for aluminum and iron stainings (Fig. 1b,c). In case 3, deposition of aluminum was focal and light, and faint positive lines were found in bony tissues (Fig. 3a). Iron staining was similar to that for aluminum in case 2 (Fig. 2b). In case 3, only focal linear reaction was observed (Fig. 3b), as on aluminum staining.

In case 1, not only bony tissues but also elastic fibers in blood vessels were black in color (Fig. 4a) and positive for both aluminum and iron (Fig. 4b,c). Positive reactions in elastic fibers for aluminum and iron were identical in pattern. Fragmentation of altered elastic fibers following metal deposition appeared to have been the cause of hemorrhage, since severe hemorrhage was always present around these vessels. Interestingly, iron-positive elastic fibers had been engulfed by multinucleated giant cells in regions of foreign-body reaction adjoining blood vessels (Fig. 4d), i.e., endogenous pneumoconiosis. In case 2, although some hemorrhage had occurred in association with siderophages, elastic fibers were completely negative for both stains, as in case 3 as well. The fine elastic fibers of alveolar septa were also positive for both aluminum and iron, in a fine network-like pattern (Fig. 4b,c) associated with marked hemorrhage, in which many iron- positive siderophages were found. Reaction for aluminum and iron was also detected in the outer rim of bronchial cartilage in case 1.

Discussion

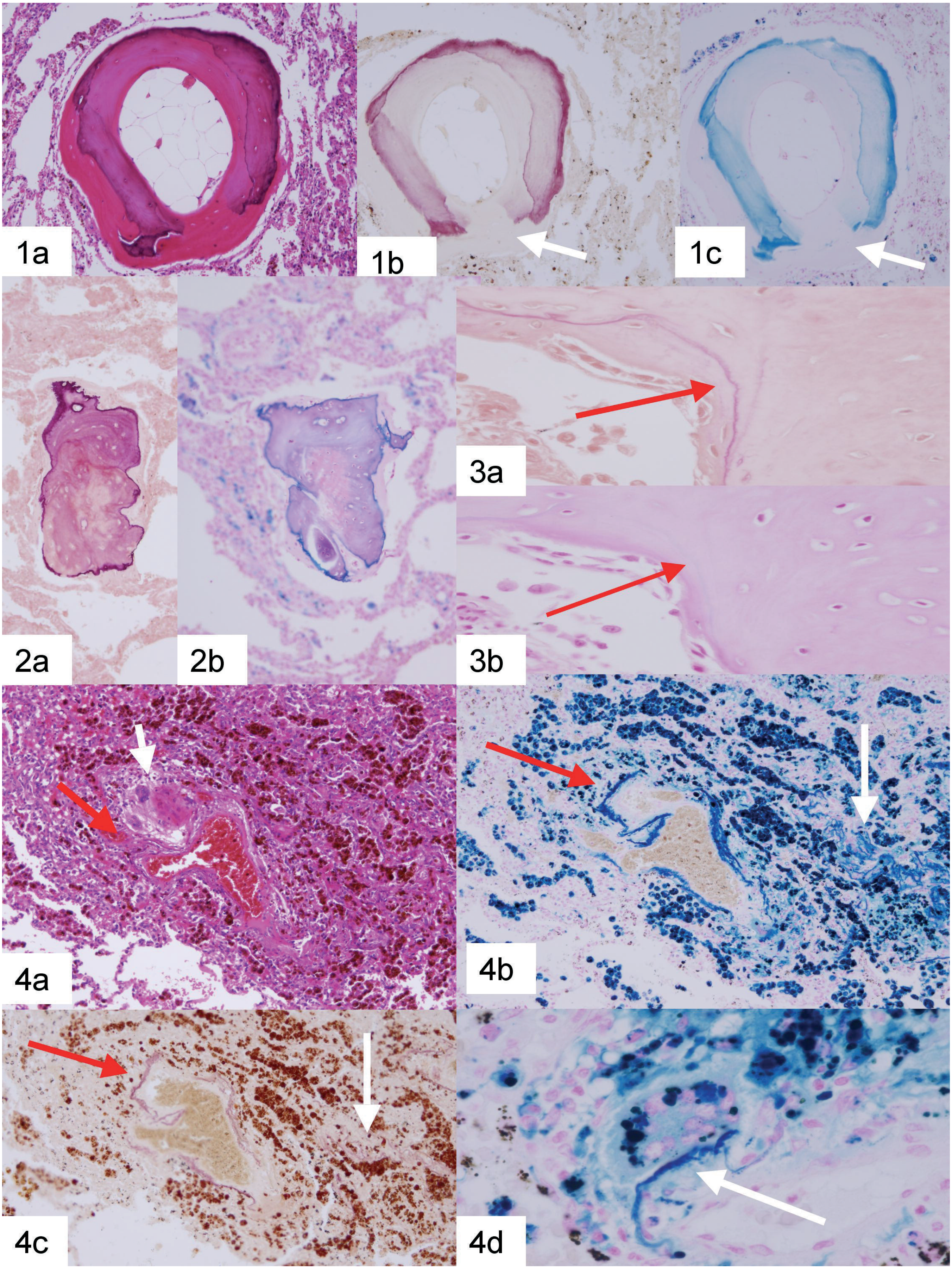
DPO is a rare condition, with a frequency reported to be 0.05% (8 of 1393cases) at autopsy (Lara et al., 2005) and 6.7% (5 of 75 cases) in cases of interstitial

Fig. 1. Bony tissue with fatty marrow exhibiting apparent dense black linear pattern **(a)** on HE stain in case 1. Both aluminum **(b)** and iron **(c)** were positive in the calcifying front of the bone. Osteoid tissues were negative on aluminum and iron stains (white arrows). x 100

Fig. 2. Linear and diffusely positive regions of staining for both aluminum **(a)** and iron **(b)** in case 2. x 100

Fig. 3. Faint linear positivity for both aluminum **(a)** and iron **(b)** at the calcifying front of bone in case 3, as indicated by arrows. x 200

Fig. 4. In a hemorrhagic region in case 1, elastic fibers of blood vessels are black in color on HE stain **(a)**. Aluminum **(b)** and iron **(c)** stains were also positive in identical regions, as indicated by red arrows. An iron-positive elastic fiber is engulfed by a multinucleated giant cell in a foreign-body reaction **(d)**, a condition termed endogenous pneumoconiosis. Fine elastic fibers are also positive on aluminum and iron staining (b&c, white arrows). a-c, x 200, d, x 400


1a
1b
1c
2a
2b
3a
3b
4a
4b
4c
4d

pneumonia (Kim et al., 2005). It is usually diagnosed at autopsy, just like cases 2 and 3 in our study , and is difficult to clearly diagnose clinically, since no ossification is found on X-ray films or routine CT studies. In the present study, we examined three cases of DPO in Japan, in order to determine the deposition of metals in the bony tissues, such as aluminum and iron. For aluminum staining, we devised a method using dewaxed sections (Ohtsuki et al., 1989) instead of the methyl methacrylate embedding method (Maloney et al., 1982). As for aluminum staining on dewaxed sections, its specificity was confirmed by using paraffin-embedded alumigel granules and methyl methacrylate-embedded definitely aluminum-positive sections as control (Ohtsuki et al., 1989). If we use decalcification processes of bony tissues, the staining results were somewhat unstable in case of over-decalcification. However, in the present study, we did not use decalcification processes in all of the three cases, therefore, we believe each positive stain is specific for aluminum. In addition, aluminum staining is reported to be the best method for demonstrating the existence of aluminum (dos Reis et al., 1997). Using this simple method (Ohtsuki et al., 1989), we demonstrated the presence of aluminum and iron in all three cases histochemically. The deposition of these metals was very similar to that in bony tissues from hemodialysis patients previously reported (Ohtsuki et al., 1989, 1992). However, in bony tissues of hemodialysis patients, no aluminum or iron deposition in elastic fibers of blood vessels was found at all. In another study, bone formation was found in the lung in 4 of 29 long-term hemodialysis patients (Bestetti-Bosisio et al., 1984), and calcium, potassium, and magnesium were detected in those pulmonary bony tissues (Bestetti-Bosisio et al., 1984; Walker et al., 1989). In particular, iron deposition in elastic fibers of blood vessels has already been reported in patients with renal or cardiac failure (Pai et al., 1994), in some cases with formation of granulomas as the result of elastophagia. The elastic fibers altered by metal deposition were fragmented, with induction of multinucleated giant cell reaction in foreign-body granulomas as detected in case 1. This phenomenon has been termed endogenous pneumoconiosis (Kaplan and Walford, 1957). These findings have also been obtained in hemosiderosis manifesting as elastogranuloma (Ziegan and Tatterman, 1967). Iron deposition, as well as that of calcium has been reported in elastic fibers of pulmonary tissues from patients with renal or cardiac failure (Pai et al., 1994). This type of deposition involved non-specific precipitation of altered elastic fibers, termed mineralizing elastosis (Pai et al., 1994).

In our previous studies, aluminum and iron deposition were found in identical locations, indicating the presence of calcifying fronts or cement lines, using a double-staining method for iron and aluminum (Ohtsuki et al., 1992). Simultaneous deposition was noted in 55 of 90 samples examined (61%) (Ohtsuki et al., 1992). On testing for aluminum deposition, 38 of 55 samples were positive for linear positive reaction in bone from hemodialysis patients in histochemical sections embedded in methacrylate and electron probe X-ray microanalysis (Boyce et al., 1992). Although aluminum and iron deposition has been demonstrated histochemically in the iliac bone of hemodialysis patients using the dewaxed section method (Ohtsuki et al., 1989, 1992) and with the methacrylate embedding method (Pierides and Myli, 1984; Van de Vyver et al., 1984; Verbueken et al., 1984), the present report is the first on aluminum deposition in pulmonary bones and elastic fibers of blood vessels in patients with DPO. These metallic depositions were not the genesis of pulmonary abnormal ossification, but the result during calcification processes in ectopic bone formation. We need to investigate the detailed mechanism of metallic deposition, such as iron and aluminum, of the bone from patients with hemodialysis, but no definite explanation has been made in the literature yet. So, metallic deposition of the bone is a well-known fact in patients with renal failure under hemodialysis (Pierides and Myli, 1984; Van de Vyver et al., 1984, Verbueken et al., 1984; Ohtsuki et al., 1989). In three patients in the present study, they did not receive any therapy of hemodialysis. We think that in patients with renal failure under hemodialysis, the abnormal metallic deposition occurred during the calcification in osteogenesis, but in the present case, abnormal metallic deposition was detected in ectopic abnormal pulmonary ossification. Although the detailed mechanism of metallic deposition is unknown, metallic deposition of iron and aluminum in newly-formed ectopic pulmonary bony tissues was demonstrated as fact. We think this metallic deposition might be one of the common phenomenon between osteogenesis under hemodialysis and abnormal pulmonary ossification, and also among all of DPO cases, in varying ages. On the histogenesis of abnormal pulmonary ossification, we can only speculate that the main factor might be chronic ischemia of pulmonary terminal air-way region, caused by cardiac failure. The detailed mechanism of metallic deposition should be investigated in future. Further studies in accumulated cases is needed for detailed analyses.

Acknowledgements. The authors are grateful for the excellent technical assistance of Mr. T. Yamaguchi, Mr. Y. Okada, Mr. T. Watanabe, Ms. M. Izumimoto, and Ms. Y. Teratani, and also for the secretarial work of Ms. M. Ohkochi, Ms. K. Yamamoto, and Ms. K. Takasuka.

References

Bestetti-Bosisio M., Cotelli F., Schiaffino E., Sorgato G. and Schmid C. (1984). Lung calcification in long-term dialysed patients: a light and electronmicroscopic study. Histopathology 8, 69-79.

Boyce B.F., Byars J., McWilliams S., Mocan M.Z., Elder H.Y., Boyle I.T. and Junor B.J. (1992). Histological and electron microprobe studies of mineralisation in aluminum-related osteomalacia. J. Clin. Pathol. 45, 502-508.

dos Reis L.M., Tzanno-Martins C. and Jorgetti V. (1997). Histochemical staining of bone aluminum: comparison of aluminon and acid solochrome azurine and their correlation with bone aluminum content. Rev. Hosp. Clin. Fac. Med. Sao Paulo 52,171-174.

Kaplan L. and Walford R.L. (1957). Pulmonary fibrosis and giant-cell reaction with altered elastic tissue; endogenous pneumoconiosis. AMA. Arch. Pathol. 63,75-90.

Kim T.S., Han J., Chung M.P., Chung M.J. and Choi Y.S. (2005). Disseminated dendriform pulmonary ossification associated with usual interstitial pneumonia: incidence and thin-section CT-pathologic correlation. Eur. Radiol. 15, 1581-1585.

Lara J.F., Catroppo J.F., Kim D.U. and da Costa D. (2005). Dendriform pulmonary ossification, a form of diffuse pulmonary ossification: report of a 26-year autopsy experience. Arch. Pathol. Lab. Med. 129, 348-353.

Maloney N.A., Ott S.M., Alfrey A.C., Miller N.L., Coburn J.W. and Sherrard D.J. (1982). Histological quantitation of aluminum in iliac bone from patients with renal failure. J. Lab. Clin. Med. 99, 206-216.

Ohtsuki Y., Yamaguchi T., Sonobe H., Takahashi K., Hayashi K., Takenaka A., Hashimoto H., Kuwabara K., Miyamoto T. and Terao N. (1989). A simplified aluminum stain in paraffin sections of bone from hemodialysis patients. Stain Tech. 64, 55-59.

Ohtsuki Y., Furihata M., Yamaguchi T., Sonobe H., Hashimoto H., Yuasa K., Takenaka A. and Terao N. (1992). Histopathological investigation on the aluminum and iron deposition of the bone in the hemodialysis patients. Kidney Metab. Bone Dis. 5, 87-92(article in Japanese with English abstract).

Ohyama H., Yamanegi K., Yamada N., Watanabe H., Terada N. and Nakasho K. (2006). A case of diffuse pulmonary ossification. Jpn. J. Diagn. Pathol. 23, 266-270 (article in Japanese).

Pai U., McMahon J. and Tomashefski J.F. Jr (1994). Mineralizing pulmonary elastosis in chronic cardiac failure. ”Endogenous pneumoconiosis” revisited. Am. J. Clin. Pathol. 101, 22-28.

Pierides A.M. and Myli M.P. (1984). Iron and aluminum osteomalacia in hemodialysis patients. N. Engl. J. Med. 310, 323.

Tseung J. and Duflou J. (2006). Diffuse pulmonary ossification:an uncommon incidental autopsy finding. Pathology 38, 45-48.

Van de Vyver F.L., Verbueken A.H., Visser W.J., Van Grieken R.E. and De Broe M.E. (1984). Localization of aluminum and iron by histochemical and laser microprobe mass analytical techniques in bone marrow cells of chronic hemodialysis patients. J. Clin. Pathol. 37, 837-838.

Verbueken A.H., Van de Vyver F.L., Van Grieken R.E., Paulus G.J., Visser W.J., D’Haese P. and De Broe M.E. (1984). Ultrastructural localization of aluminum in patients with dialysis-associated osteomalacia. Clin. Chem. 30,763-768.

Walker E.R., Frederickson R.G. and Mayes M.D. (1989). The mineralization of elastic fibers and alterations of extracellular matrix in pseudoxanthoma elasticum. Ultrastructure, immunocytochemistry, and X-ray analysis. Arch. Dermatol. 125, 70-76.

Ziegan J. and Tattermann G. (1967). Elastogranulomas in idiopathic lung hemosiderosis. Zentralbl. Allg. Pathol. 110, 433-441 (article in German).

Accepted July 18, 2007