

CASE STUDY

Pulmonary fibrosis and sea-blue histiocyte infiltration in a patient with primary myelofibrosis

K. Yamauchi*, K. Shimamura**

Pulmonary fibrosis and sea-blue histiocyte infiltration in a patient with primary myelofibrosis. K. Yamauchi, K. Shimamura. ©ERS Journals Ltd 1995.

ABSTRACT: The authors present the case of a 73 year old man with primary myelofibrosis, pulmonary fibrosis, and acquired sea-blue histiocytosis, who died of respiratory failure.

Pathology of the lungs revealed infiltration by sea-blue histiocytes, and fibrosis in the alveolar septa, and clumps of these cells filling the alveolar spaces. Megakaryocytes were also occasionally observed in the alveolar capillaries.

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Depts of *Internal Medicine and **Pathology, School of Medicine, Tokai University, Japan.

Correspondence: K. Yamauchi, Dept of Internal Medicine, Tokai University School of Medicine, Bohseidai, Isehara-shi, Kanagawa-ken, 259-11, Japan

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In patients with myelofibrosis, extramedullary haematopoiesis commonly occurs in the spleen and liver, but rarely in the lymph nodes [1]. In these organs, fibrosis frequently develops in association with megakaryocyte infiltration [2].

On the other hand, macrophages loaded with cytoplasmic granules staining blue with Giemsa, so-called sea-blue histiocytes, are commonly seen in various organs including the lungs in Niemann-Pick disease, and occasionally in the bone marrow and spleen as a secondary phenomenon associated with a variety of acquired haematological disorders, such as chronic myelogenous leukaemia, chronic immune thrombocytopenic purpura, myelodysplastic syndrome, and polycythaemia rubra vera [3–5]. Pulmonary involvement is a complication occurring frequently in the inherited disorder [6, 7], but rarely in acquired disorders.

Case report

A 73 year old man was admitted for an evaluation of abdominal extension and progressive general fatigue of 5 months duration. Physical examination revealed anaemic conjunctiva, supraclavicular lymph node swelling, a mild mid-systolic murmur of the heart, and normal respiratory sounds. The liver was palpable 4.5 cm below the right costal margin, and the spleen was palpable 4.5 cm below the umbilicus.

On admission, haematological data included the following: a white blood cell count (WBC) of 5.7×10^9 cells·L⁻¹, including 86% neutrophils, 6% lymphocytes, 5% eosinophils, 2% monocytes and 1% metamyelocytes; a haemoglobin level of 5.6 g·dL⁻¹; and a platelet count of 41×10^9 platelets·L⁻¹.

A chest radiograph showed mild cardiomegaly and

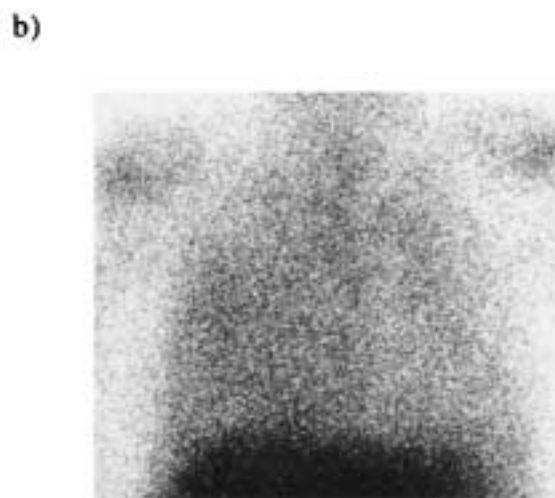
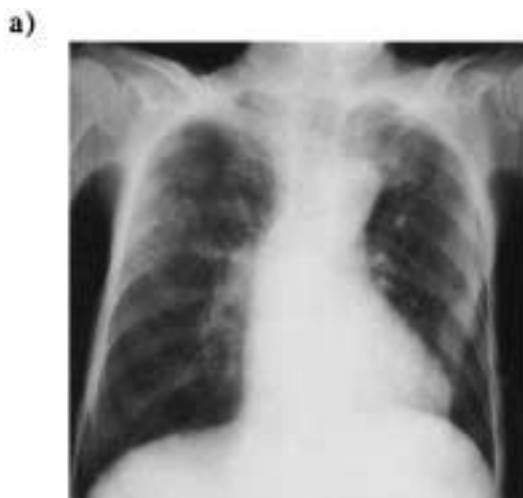


Fig. 1. – a) Chest radiograph depicting widespread reticular lesions in the lungs. b) Whole body scan with ⁶⁷gallium-citrate showing radioactive accumulation in the lungs.

marked reticular infiltration spreading diffusely throughout the lung fields, consistent with fibrosis (fig. 1a). Arterial blood gas analysis in room air showed: pH 7.48; arterial oxygen tension (P_{a,O_2}) 9.6 kPa (71.4 mmHg); and arterial carbon dioxide tension (P_{a,CO_2}) 3.3 kPa (24.8 mmHg); and bicarbonate concentration 18.6 mmol·L⁻¹. A whole body scan with ⁶⁷gallium-citrate showed mild accumulation of radioactivity in the lungs, suggesting active interstitial pneumonitis (fig. 1b).

Bone marrow aspiration and biopsy showed marked reticulin fibrosis and hypercellular marrow, with no maturation arrest of the myeloid lineage and with increased megakaryocytes (0.531×10^9 cells·L⁻¹). There was also heavy infiltration by atypical macrophages, the cytoplasm of which were distended and closely packed with fine granules staining blue with May-Grünwald Giemsa preparation. The nuclei were centrally located in some cells and displaced towards the periphery in others. These features were consistent with sea-blue histiocytes [6, 7].

Familial history of Niemann-Pick disease was absent. Chromosomal analysis of bone marrow cells did not reveal abnormal karyotype. Serum lysozyme activity was 28.0 µg·mL⁻¹ (control range 5.0–10.2 µg·mL⁻¹). Supraclavicular lymph node biopsy showed fibrosis and infiltration by the myeloid cells and megakaryocytes, as demonstrated by immunohistochemical staining of CD15 (Leu M1) and anti-von Willebrand factor antibodies, respectively, suggesting extramedullary haematopoiesis.

Oxymetholone was given to improve the progressive pancytopenia, but was not effective. Haematological data 2 weeks before death were: WBC of 5.2×10^9 cells·L⁻¹, including 68% neutrophils, 12% lymphocytes, 10% monocytes, 2% eosinophils, 1% basophils, 2% metamyelocytes and 5% myelocytes; a haemoglobin level of 4.8 g·dL⁻¹; and a platelet count of 11×10^9 platelets·L⁻¹. Fourteen months after admission, the patient died of respiratory failure and sepsis due to *Staphylococcus aureus* resistant to piperacillin, after intermittent fever continuing for 1 week. No cytotoxic drugs were administered in the course of treatment.

Autopsy findings

In formalin-fixed, paraffin-embedded sections, the bone marrow showed reticulin fibrosis and infiltration by megakaryocytes and sea-blue histiocytes, with a pale, large cytoplasm including fine granules. The histiocytes accounted for approximately 30% of the cellularity. The lungs showed not only infiltration by the histiocytes into the alveolar septa but also clumps of these cells within alveolar spaces, which resulted in obliteration of the functional air exchanging tissue and alveolar capillary vessels (fig. 2a). Some histiocytes had 2–4 nuclei and enlarged cytoplasm. The cytoplasm stained granularly with oil red O and Sudan black B, and included periodic-acid-Schiff positive materials that were digested with diastase. Immunohistochemical staining with the indirect peroxidase-labelled antibody method demonstrated

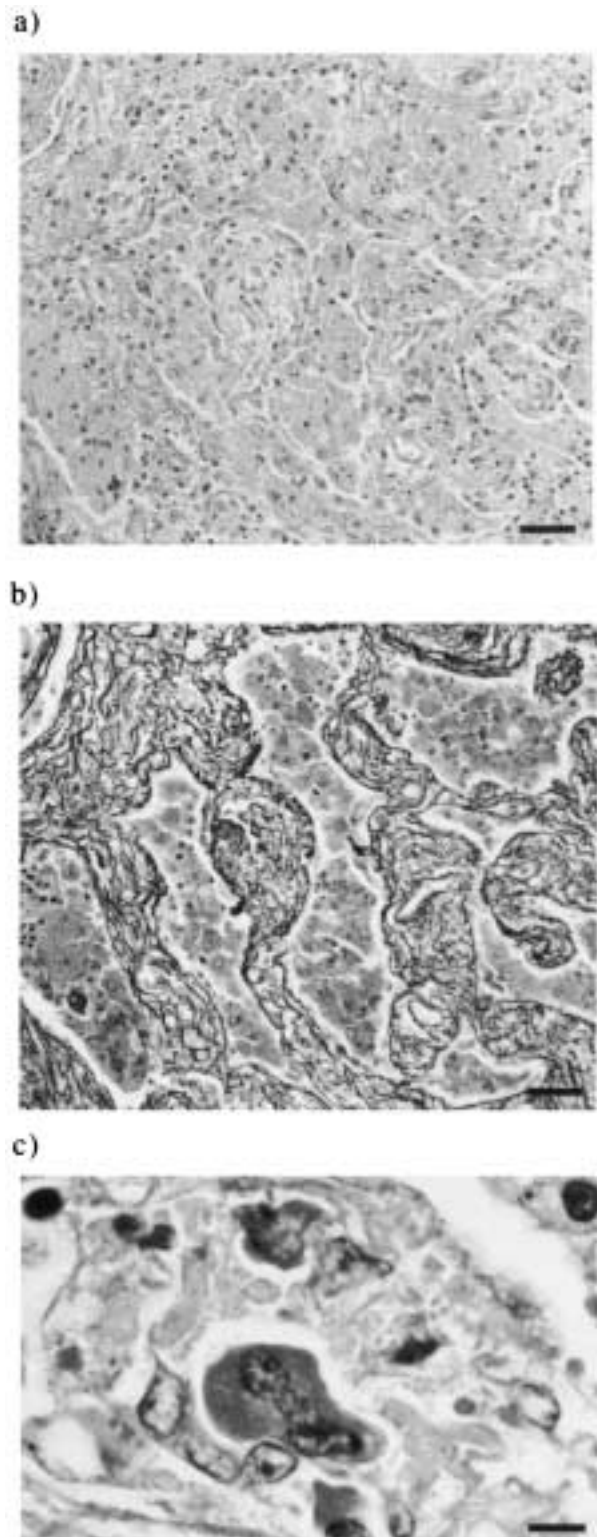


Fig. 2. – Lung autopsy sections. a) Sea-blue histiocytes are infiltrated massively into alveolar septa and spaces. Some giant histiocytes with multiple nuclei and abundant cytoplasm are also visible. (Haematoxylin and eosin staining). b) Reticulin fibres increase and surround the histiocytes infiltrated into the alveolar septa in the area shown in (a). (Gomori's silver impregnation). c) A megakaryocyte with multiple nuclei and abundant cytoplasm is visible in a capillary vessel of the alveolar septa. (Haematoxylin and eosin staining). (Internal scale bar = 50 µm in (a) and (b), and 5 µm in (c); magnification = $\times 160$ in (a) and (b), and $\times 1,600$ in (c)).

lysozyme antigen in the cytoplasm. Reticulin fibres were remarkably increased in the alveolar septa (fig. 2b). Megakaryocytes stained positively with anti-von Willebrand factor antibody were observed sporadically in the capillary vessels of alveolar septa (fig. 2c). No infection leading to respiratory failure was observed in the lungs.

The liver showed moderate infiltration by the histiocytes into sinusoids and portal areas, but no increase in reticulin fibres. The spleen showed a moderate increase in reticulin fibres and massive infiltration by the histiocytes (approximately 30% of the cellularity). The spleen also had multiple small infarcts, supposedly caused by enlarged histiocytes occluding the capillary network. Extramedullary haematopoiesis was observed in these organs. The liver and the spleen weighed 2,100 and 1,600 g, respectively. Similar infiltration by histiocytes was noted in the submucosal spaces of the small intestine wall and para-aortic nodes. Cultures of tissues obtained from the liver, spleen, and cardiac muscle, and of arterial blood in intracardium demonstrated methicillin-resistant *Staphylococcus aureus*.

Discussion

Pulmonary involvement is seen in lipid storage diseases resulting from an absolute or relative deficiency of lysosomal enzymes in the reticuloendothelial system. The inherited diseases include Gaucher's disease [8], Niemann-Pick disease [6, 7] and Hermansky-Pudlak syndrome [9]. Pulmonary diffuse reticular interstitial infiltrates, honeycombing, disseminated nodules or punctate calcification, predominating in the lower lobes, have been reported as roentgenographic findings common to these lipid storage diseases. A miliary or interstitial infiltrate was present on chest radiographs in 23% of 42 cases of sea-blue histiocytosis reviewed by QUATTRIN *et al.* [7]. Most of those patients, however, remained asymptomatic. The pathological examinations showed that foamy macrophages fill the alveoli and infiltrate the alveolar septa, with septal fibrosis occasionally observed. These findings are rare in acquired sea-blue histiocytosis.

In idiopathic pulmonary fibrosis and Hermansky-Pudlak syndrome, platelet-derived growth factor (PDGF) is released from activated macrophages and plays an important role in pulmonary fibrosis [10, 11]. In this patient, sea-blue histiocytes that infiltrated the lungs may also have released PDGF and promoted the fibrosis. Another explanation is that the ceroid-like material seen in the alveolar macrophages may play a role in inducing fibrosis [9]. When activated, macrophages undergo an increase in oxygen consumption, and secretion of superoxide anion and hydrogen peroxide. These oxygen metabolites are implicated in a variety of cellular and tissue injuries [12]. It is possible that phagocytosis of cell debris or the ceroid-like material by the macrophages leads to increased production and release of superoxide [13, 14], and these active substances may contribute to subsequent parenchymal damage. A third possible explanation is that pulmonary megakaryocytes with alpha-

granular deficiency may induce fibrosis in the lungs by the same mechanism as in the bone marrow of myelofibrosis. Megakaryocytes with abundant cytoplasm were observed in the alveolar capillary vessels of this patient.

We reported a patient with myelofibrosis secondary to chronic myelogenous leukaemia, in whom pulmonary fibrosis seems to have occurred in association with megakaryocyte infiltration of the lungs [15]. Studies of the physiological kinetics of megakaryocytes reveal that the cells that differentiated in the bone marrow are transferred through caval veins to the lungs and caught in the capillary bed because of their bulk [16, 17]. Fibrosis occurring in the bone marrow of patients with chronic myeloproliferative disorders is considered to be caused by growth factors, including PDGF, that are spontaneously released from marrow megakaryocytes with alpha-granular deficiency [18]. In the lungs of this patient, PDGF may have been spontaneously released from megakaryocytes caught in the pulmonary vasculature and have induced fibrosis.

Within the spleen, which in myelofibrosis functions as an extramedullary haematopoietic organ, monocytes differentiate from haematopoietic stem cells, proliferate, and are transformed into sea-blue histiocytes. The monocytes are transferred through the splenic vein to the liver. Because sea-blue histiocytes have a large cytoplasm, as do megakaryocytes, it is considered likely that the lungs, and probably the liver, function as a physiological sieve of circulating monocytes, which changed into sea-blue histiocytes in the bone marrow and the spleen, respectively. Moreover, it has been reported that, in a labelling experiment with ³H-thymidine, about 15 and 56% of mice monocytes leaving the circulation become pulmonary macrophages and Kupffer cells, respectively [19]. Consequently, a heavy accumulation of sea-blue histiocytes in the lungs and the liver of this patient could be explained by these hypotheses.

Excessive cell destruction is caused by either dyshaematopoiesis occurring in myelodysplastic syndrome, or autoantibody to platelets in immune thrombocytopenic purpura. A marked proliferation of haematopoietic cells in chronic myeloproliferative disorders leads to increased turnover. The lysosomal enzyme pathway in macrophages of these disorders results in saturation for removal of the membrane lipid, and the cells are transformed into sea-blue histiocytes [3-5]. The sea-blue histiocytes are usually observed in the bone marrow and spleen, where the majority of the blood cells are destroyed and phagocytosed. On the other hand, LINKS *et al.* [20] reported a case of pulmonary tuberculosis in which pulmonary interstitial infiltration of pseudo-Gaucher cells was caused by incomplete breakdown of mycobacterial cell wall. In our patient with primary myelofibrosis, macrophages belonging to the malignant clone probably had defective catabolic enzymes, and were transformed into the sea-blue histiocytes as the cells phagocytosed *Staphylococcus aureus* that disseminated in various organs.

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