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Lung disease and iron overload

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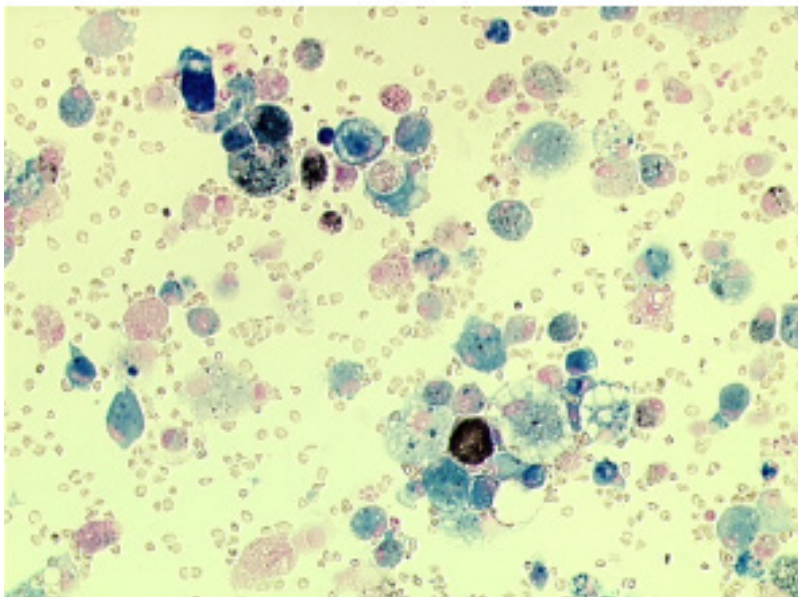
Notes are linked to the references page.

The literature reports that the pulmonary build-up of haemosiderin without any evidence of past bleeding is seen quite often but, unless it is particularly heavy, clinicians rarely take much notice of it. It is important to emphasise that, as a result of a common terminological misuse, the term idiopathic haemosiderosis is often used by lung specialists as a synonym for idiopathic alveolar bleeding.

BRONCHO-ALVEOLAR IRON OVERLOAD

Lung specialists can measure the amount of iron in the lung by collecting macrophages by broncho-alveolar lavage and estimating their average iron content by Perls staining (Figure 1.). Staining makes it possible to work out the percentage of macrophages loaded with haemosiderin and a result of over 30% is considered pathological. The Golde score — a semi-quantitative estimate of haemosiderin content in 100 macrophages — is also calculated from Perls-stained sections: every cell is scored at between 0 and 4 for haemosiderin content and a score of over 100 is considered pathological.

Figure 1. Perls-stained macrophages collected by broncho-alveolar lavage



Macrophages containing haemosiderin stain blue with Perls. ©Dr Homa ADLE.

The investigation of occult bleeding relies on evidence from experiments like those of Epstein et *al*.:[1] blood was injected into the airways of mice and then broncho-alveolar lavage is performed at a series of different time points. The build-up of haemosiderin inside alveolar macrophages was detected three days after inhalation, peaked on day 7 and persisted for two months. The investigators also observed the accumulation of lymphocytes and neutrophils in the alveoli, peaking two days after inhalation of the blood and persisting for two months. The presence of blood in the lung is therefore not neutral in that it clearly affects local cell populations although it is not possible to determine what happens to the iron or red blood cells.

CASE HISTORY: IRON OVERLOAD IS NOT ALWAYS ASSOCIATED WITH OCCULT BLEEDING

Occult bleeding is the most common cause of haemosiderin build-up in alveolar macrophages. Occult bleeding can have various aetiologies, the most common being left heart failure.[2] The case history presented here shows how lung specialists try to identify the reason for iron build-up in macrophages, probably pointing to occult bleeding.

A colleague of the author referred a 46-year-old man with chronic alveolar haemorrhage. A computed tomography (CT) scan showed hyperdense, ground glass centrolobular foci, especially in the upper lobes. Broncho-alveolar lavage revealed massive iron accumulation in the alveolar macrophages. The Golde score was 186 and nearly all of the macrophages contained haemosiderin.

An exhaustive investigation of known causes of intra-alveolar bleeding did not reveal anything. A Perls-stained transbronchial biopsy showed macrophages and also epithelial cells loaded with iron. Analysis of a surgical lung biopsy confirmed the iron build-up in macrophages and epithelial cells. Analysis of the particulate content of the lung indicated a particle count well above that in the population as a whole, but quantitation was impossible because of the heavy load of iron particles, ‘probably endogenous’ in origin, according to the report.

Interestingly, the report also described the presence of abnormally high levels of aluminium, manganese, nickel and chrome.

The patient explained that he had worked as an arc welder for 26 years in the boiler-making industry so he had been exposed to particle-laden gases in a confined environment for nearly three decades, including iron particles which are formed in the course of welding.
An iron work-up showed high blood ferritin with normal serum iron and oxygen saturation. Magnetic resonance imaging (MRI) of the liver showed increased iron load.

Finally, a diagnosis of pulmonary siderosis was suggested. Pulmonary siderosis corresponds to iron build-up inside macrophages and is mainly seen in welders and other workers who handle iron, e.g. steelmakers, foundry workers, etc. The siderosis is benign and does not progress to fibrosis. Fibrosis is initiated when iron is not the only causal factor; typically, the inhalation of both iron particles and silica leads to silicosiderosis.[3, 5]

In our case history, the patient’s lungs had been exposed to iron for 26 years without any significant functional impairment or progression to fibrosis. His only symptom (which had motivated the visit to the lung specialist) was occupational asthma. This regressed when he stopped working and the hyperdense, ground glass parenchymal foci steadily regressed.

SIDEROSIS IN THE LITERATURE

The literature provides a little information about pulmonary siderosis. CT scanning shows poorly defined centrolobular nodules and hyperdense ground glass foci.[5, 6] The centrolobular localisation of the nodules bears witness to the role of inhalation in pathogenesis. Irregular—rough, dark brown—particles of iron oxide and a variable number of particles of haemosiderin build up inside macrophages, mainly around the bronchi and

vessels. In extreme cases, the pulmonary parenchyma can be rust-coloured or brown. Fibrosis is usually limited.[4, 7]

The only remedy is to stop the exposure which will lead to some regression of the shadows, although in some cases broncho-alveolar lavage has been attempted to try to speed up resorption of the built-up iron.[4, 8]

A number of studies have reported high ferritin levels in welders.[9, 11] These workers are constantly inhaling vapours containing a mixture of nanoparticles of different metals. They can develop frank pathology if they are also exposed to silica, asbestos or tobacco smoke.[3, 12, 13] A French study on sections of lung biopsy material taken from welders found a build-up of nanoparticles associated with welding (mainly oxides of iron, manganese and chromium) inside macrophages in the alveolar cavity and fibrous regions.[7]

PULMONARY MANIFESTATIONS IN BETA-THALASSAEMIA

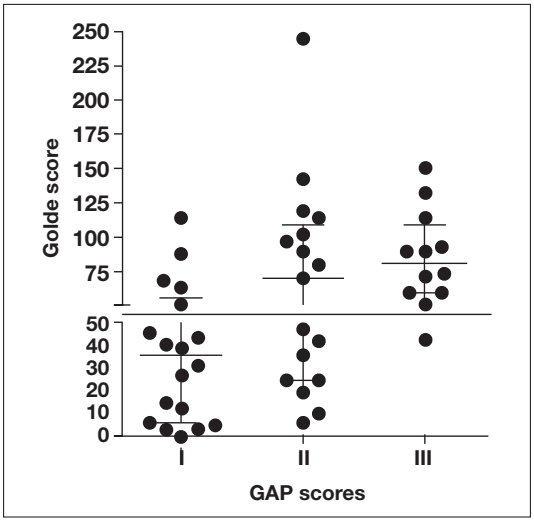
According to the literature, beta-thalassaemia is associated with restrictive respiratory problems which could be proportionate to the iron overload, although this idea has recently been questioned. Beta-thalassaemia and the various forms of haemolytic anaemia might be associated with an increased incidence of precapillary pulmonary hypertension although no link has been defined with iron overload. Finally, there has been at least one report of pulmonary fibrosis in a patient with beta-thalassaemia although the possibility of the same person suffering from two unrelated diseases cannot be ruled out.[14, 17]

IRON OVERLOAD AND PULMONARY FIBROSIS

Links between fibrosis and iron overload have been investigated in a series of studies. Comparison of iron build-up inside alveolar macrophages as measured by the Golde score in cases of idiopathic pulmonary fibrosis (IPF) and age-matched controls showed that if they remained within the normal range with scores of below 100 in patients with fibrosis, Golde scores were nevertheless significantly higher than in controls (54.0±43.4 and 10.5±13.2; $P<0.02$).[18]

Histological studies have also shown excessive iron build-up in patients with IPF.[19] Another showed exaggerated iron accumulation in broncho-alveolar fluid and alveolar cells, unrelated to smoking. Differences between Golde scores for alveolar macrophages from current smokers and people who have never smoked were not significantly different ($P=0.53$). The GAP mortality score assesses prognosis in patients with IPF.[20] This study showed tight correlation between macrophage iron load and the severity of pulmonary fibrosis as predicted by a prognostic GAP score (Figure 2.).[21]

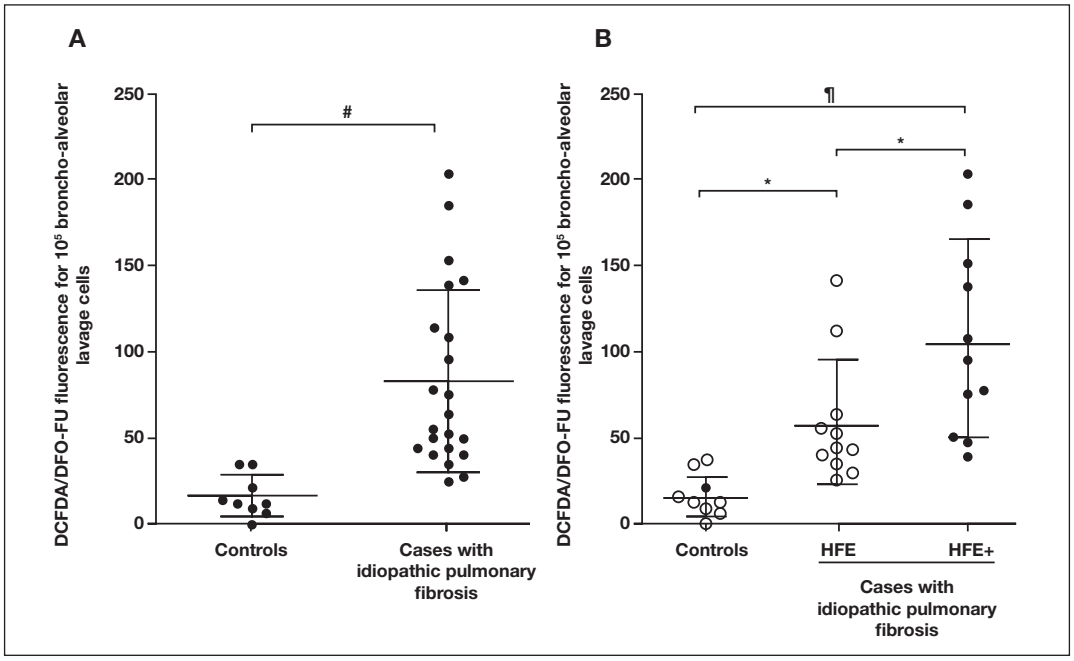
Figure 2. Correlation between iron load and IPF severity[21]



The Golde scores of patients with IPF were classified according to disease stage (GAP I, II and III). The horizontal line on the y-axis indicates the upper limit of normal for the Golde score. © Puxeddu

The same group showed that the frequency of variant HFE alleles (C282Y, S65C and H63D) was markedly higher in cases with IPF than in controls and that this was associated with the increased generation of iron-dependent reactive oxygen intermediates. This might suggest that the dysregulation of iron metabolism as a result of genetic abnormality could play a significant role in increased susceptibility to environmental factors (Figure 3.).[18]

Figure 3. Generation of iron-dependent reactive oxygen intermediates and the frequency of variant HFE alleles in patients with IPF[18]



(a) Quantities of deferroxamine-chelatable and 5-(and-6)-chloromethyl-2′,7′-dichloro-dihydrofluorescein diacetate acetyl ester (CM-H2DCFDA) induced by iron-dependent reactive oxygen intermediates generated by unstimulated broncho-alveolar lavage cells from healthy controls and cases with IPF. Fluorescence intensity is expressed as DCFDA/DFO-FU per 105 broncho-alveolar lavage cells.
(b) Stratified data according to the expression of variant HFE alleles. Controls and cases with IPF (*HFE*-) homozygous for the wild-type *HFE* sequence are shown as empty dots, and controls and cases with IPF (HFE +) expressing variant HFE alleles are shown as solid dots. Means ± 1 standard deviation are shown as horizontal lines and the vertical lines represent, respectively #P<0.0001; ¶P<0.005; *P<0.05.
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So it seems that a series of links exists between iron metabolism and pulmonary fibrosis, but could repeated bloodletting predispose to the progression to pulmonary fibrosis?

Idiopathic pulmonary haemosiderosis (IPH) is a rare cause of alveolar haemorrhage in children. The French paediatric IPH cohort has 25 cases. Chest imaging detected diffuse parenchymatous infiltration in half of these young patients. The diagnosis was established on the basis of the detection of haemosiderin-laden macrophages in either broncho-alveolar lavage fluid (19/25 cases) or a lung biopsy (6/25). All the patients were first started on corticosteroids. In 13 cases, immunosuppressive agents were prescribed because of corticoresistance or serious adverse reactions. The median follow-up time was 5.5 years, with a satisfactory respiratory outcome in 23/25 patients although one patient developed severe pulmonary fibrosis.[22]

Was the fibrosis induced by the chronic bleeding or does the disease explain the haemorrhage? This question has no clear answer yet but it seems that iron build-up can in and of itself cause pulmonary fibrosis.

IRON OVERLOAD AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) is extremely common, mainly because of the prevalence of smoking, although it is known that it can have other causes.

In a recent study,[23] lung tissue was taken from donors and the recipients of transplanted lungs for COPD (GOLD 2–3 and GOLD 4). Broncho-alveolar lavage cells were obtained from non-smokers, healthy smokers and patients with COPD (GOLD 1–3). Iron-laden cells were quantitated by histological analysis (Perls staining) and the expression of genes related to iron sequestration (transferrin and transferrin receptor), storage (ferritin) and export (ferroportin) and was measured by real-time polymerase chain reaction (PCR).

This work showed that iron deposition and the percentage of macrophages that contained iron increased with the severity of the COPD and emphysema. The expression of transferrin and ferritin mRNAs was significantly higher in the lungs of patients with COPD (GOLD 4) than in the donors (by factors of 6.9 and 3.22, respectively). In the broncho-alveolar lavage cells, the expression of transferrin, transferrin receptor and ferritin mRNAs correlated with the degree of obstruction of the airways.

These findings point to a link—but not a causal relationship—between pulmonary iron overload and the disease. The investigators saw the sequestration of iron by alveolar macrophages as a protective mechanism against iron-induced oxidative stress.

Another experiment[24] conducted in mice showed that smoking induces a build-up of iron in mitochondria. In particular, it was shown that an iron-depleted diet or the administration of a chelating agent protected the animals against the deleterious effects of tobacco smoke. This could point to a causal link between iron overload and the development of bronchial disease and emphysema.

CONCLUSION

The physiopathological role of iron overload in chronic respiratory disease remains unclear but there is now a certain amount of evidence to indicate that the question warrants further investigation.

For the lung specialist, iron overload remains a diagnostic tool for the identification of chronic alveolar haemorrhage even if the possibility of some exogenous source of iron has to be considered.

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