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Black Spots of the Parietal Pleura: Morphology and Formal Pathogenesis

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Key Words
Black spots · Pleura · Coal dust · Asbestos · Mesothelioma

Abstract
Background: Dark incorporations (black spots) have been described in various organs. 'Black spots' seen as flat-to-nodular lesions of the parietal pleura are common findings in former miners. They represent areas of coal dust accumulation. An increased incorporation of asbestos fibres has been described in these areas, causing them to be seen as potential starting points for malignant mesotheliomas. ***Objectives:*** The aim of our examinations was to describe the morphology of black spots in order to understand their formal pathogenesis and discuss their role in the development of malignant mesotheliomas. ***Materials:*** We report the results of the morphological and energy dispersive X-ray analysis of 12 black spots (4 surgical and 8 autopsy specimens) located in the parietal pleura. ***Results:*** Black spots of the pleura develop in close correlation to lymphatic channels and blood vessels. Their formal pathogenesis is characterized by a mild fibrosis and an inflammatory reaction to the incorporated foreign particles. The proliferation of connective tissue may result in the formation of hyaline granulomas. Aluminum, silicone and sometimes fibres are also found in such areas. Mesothelial cells may be irritated. ***Conclusion:*** Although there are hints for an increased proliferation of mesothelial cells in some areas with black spots, our findings do not support the classification of black spots as an obligate early lesion in the development of malignant mesotheliomas.

Introduction

The term 'black spots' refers to dark, circumscribed pigment incorporations or black foci of different origin found in various organs [1]. Such black foci in the parietal pleura resulting from incorporations of mixed dust pigments were first described over a hundred years ago [2] and are commonly encountered in autopsy specimens from former coal miners. In 1867, Zenker [3] associated 'black spots' in the parietal pleura with prolonged occupational mixed dust exposure. Other authors regard black spots as the morphological correlate of pulmonary anthraco-silicosis [4]. Intrapleural mixed dust particle accumulations in the region of pleural lymphatic channels ensue from inhalation of foreign substances after prolonged occupational dust exposure.

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Boutin et al. [5] discussed the role of black spots as accumulation sites for asbestos fibres and consequently focused on them as potential starting points for malignant mesotheliomas. Compared to normal pleural regions they regarded black spots containing asbestos fibres as probable pleural target zones for the development of mesotheliomas and/or pleural plaques.

Black spots are also frequently found in the parietal pleura of former coal miners in the German Ruhr region without a concomitant rise in the incidence of non-asbestos-related mesotheliomas.

The aim of our study was to understand the formal pathogenesis of black spots and their possible role in the development of mesotheliomas. There are hints for an increased proliferation of mesothelial cells in areas closely related to dust accumulations in black spots.

An exact morphological characterization of black spots by means of energy dispersive X-ray microanalysis allows the differentiation of mixed dust accumulations from other causes of inflammatory pulmonary lesions.

Material and Methods

We report the results of the morphological, histological, ultrastructural and energy dispersive X-ray analysis of 12 patients with black spots (4 surgical and 8 autopsy specimens) located in the parietal pleura.

The specimens were fixated in 5% formaldehyde to stabilize organic structures. After standard preparation they were examined under the light microscope, combined with immunohistochemical marking with antibodies against calretinin (polyclonal antibody, Swant, 1:3,000), B-(CD20, monoclonal antibody, Dako, 1:800) and T-lymphocytes (CD45RO, monoclonal antibody, Dako, 1:400), staining machine: 'Tech Mate 2000', Dako.

Specimens for scanning electron microscopic investigations were cut, mounted onto Thermanoc slides and coated with coal (40 nm, Edwards sputter coater) and examined with a scanning electron microscope (Zeiss/LEO DSM 940) combined with an energy dispersive X-ray analysis system (Oxford, Link system, AN 10/25).

EDX- analysis provides a method to identify elements with high sensitivity and precise location in scanning electron microscopy and transmission electron microscopy. With this method, X-rays generated by the interaction of the primary beam with the specimen are sorted electronically and give information about the elemental composition of the specimen.

For transmission electron microscopy black spots were fixed with 2.5% glutaraldehyde, dehydrated, and embedded in Durcupan. Ultrathin sections were stained with Ultrostain I and Ultrostain II (Leica). A transmission electron microscope (Zeiss/LEO, EM 902) combined with an EELS analysis system was additionally used to characterize mixed dust pigment incorporations (TEM: delta E = 0, no ESI mode, 50 kV).

Qualitative EELS analysis (energy element loss spectroscopy) can be used to show the presence of elements in an image. When accelerated electrons are transmitted through the specimen some of them are scattered. In EELS the primary processes of electron excitation is directly studied and give information about elemental composition of the investigated probe.

Results

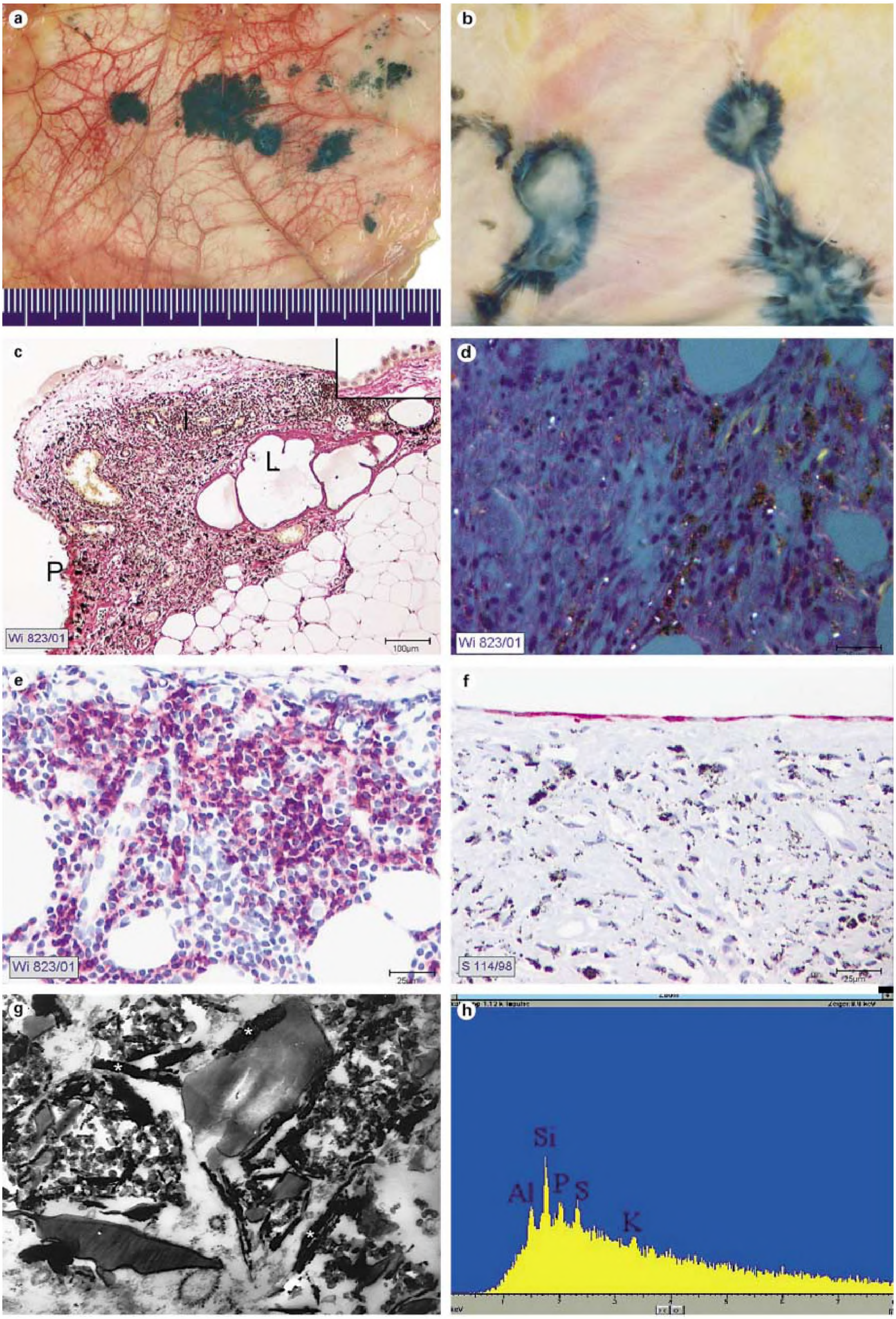
Macroscopic and Light-Microscopic Findings

Macroscopically, black spots appear as flat, punctate or planar dark pigmentations along the lymphatic channels in the parietal pleura (fig. 1a). Size and shape are subject to variation ranging from round/oval with a diameter of 2 × 2 mm to 3 × 0.2 cm in size. They occur bilaterally either singly or in clusters and are mainly located in the middle or lower lateral and occasionally sternal zones of the thoracic pleura. In rare cases, the entire parietal pleura can be covered by small black spots. Black spots are limited to the parietal pleura without invasion of the surrounding thoracic wall structures. Adhesions to the opposite visceral pleura are uncommon. The surface of the black spots is generally smooth. During the preparation process they can be easily removed from their undersurface together with an adherent piece of pleura. The vast majority of black spots are only slightly raised ranging in thickness from 0.5 to 1 mm. Nodular black spots ranging in thickness from 2.5 to 4 mm (fig. 1b) are rather rare findings.

With careful preparation, the surface of black spots remains covered by an intact mesothelial cell layer. The mesothelial cells covering nodular black spots can focally lose their flat shape, hypertrophy and proliferate.

Accumulations of mixed dust pigments contiguous with lymphatic channels are seen embedded in the pleural stroma (fig. 1c).

Fig. 1. a Macroscopic view of black spots in parietal pleura. **b** Nodular black spots with central and peripheral scar formations. **c** Light microscopy of the parietal pleura with mixed dust pigment incorporation (P), ectasy of lymphatic channels (L), inflammation (I) and reactive proliferation of the mesothelial cell (inset). **d** Polarization microscopy. Light refracting particles in the areas of mixed dust accumulation. **e** B lymphocytes (red) in a black spot marked with CD20 antibodies. **f** Mesothelial cells (red) on the surface of a black spot marked with calretinin. **g** TEM. Dark mixed dust accumulation in a black spot consisting of fibre-like formed silicates (*). According to EDX analysis most probably muscovite. × 12,000. Peaks for silicone and aluminum. Possible mineral is muscovite: Data based on automatically identifying computer analysis and on Rösler [9]. **h** EDX analysis of a black spot. Peaks for silicone and aluminum.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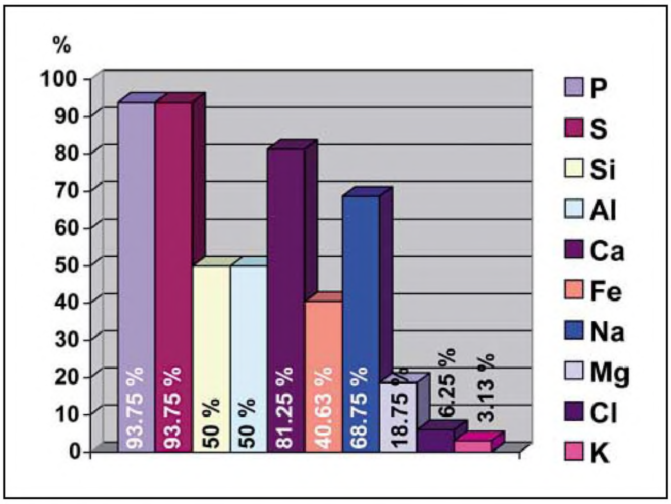

Fig. 2. Relative concentration of the elements found by means of EDX single spot analysis.

Lymphocytic infiltrates can be identified within the circumscribed portion of black spots; however, the inflammatory reaction itself is usually mild.

In nodular black spots depending on the incorporation of silicone particles there is evidently stronger fibrosis or even hyalinized granulomatosis.

Immunohistochemical Findings

Immunohistochemical markers were able to confirm the presence of T- and B-lymphocytes within black spots (fig. 1e).

Mesothelial cells on the surface of black spots undergoing hyperplastic reactive changes can be accentuated with, for example, keratin or calretinin antibodies (fig. 1f).

Electron Microscopic and Energy Dispersive X-Ray Microanalysis Findings

Transmission electron microscopy of black spots revealed in parts flattened electron-dense incorporations and in parts electron-dense inclusions interspersed between collagen fibres and adipocytes (fig. 1g, h).

EELS analysis was able to identify comparatively high silicone concentrations and in parts also traces of aluminum and carbon.

Scanning electron microscopy was used for the characterization of mixed dust incorporations. 32 single spot analyses were carried out.

Figure 2 shows the elements found and their relative concentrations. Even in adjoining areas, the element distribution turned out to be quite heterogenous comprising predominantly silicone- and aluminum-containing deposits.

Pathogenesis of Black Spots

The start and subsequent development of black spots encompasses several stages, which correlate well with the corresponding histological findings. The initial phase includes inhalation of mixed dust particles and their deposition in the parietal pleura. The inhaled particles are transported in the lymph across the pleural cavity and reach the thoracic pleura via the stomata before being deposited along the lymphatic channels. The areas where the mixed dust particles were laid down appear macroscopically as black spots. In the course of time, the incorporated dust particles trigger a low-grade inflammatory reaction of the parietal pleura. The liberation of mediators leads to fibroblast activation generating in turn a concomitant low-grade fibrosis in the main pleural layer. On rare occasions, the inflammatory pleural reactions are so pronounced that high-grade fibrotic changes of the main pleural layer ensue. The results are macroscopically nodular black spots. By means of light and scanning electron microscopy, granulomatoid structures with concentrically arranged fibres can be documented. Reactive mesothelial cell hyperplasia can be observed on the surface of the black spots. The phasic development of pleural reactions within the regions of black spots is summarized schematically in figure 3.

Black Spots and Asbestos-Induced Lesions

In some of the specimens examined, black spots occurred together or close to hyaline pleural plaques. The morphological comparison of black spots with hyaline pleural plaques revealed in both cases a marked proliferation of collagenous fibres in the main pleural layer. This was, however, the only feature these two types of lesions had in common.

The collagen fibre mesh seen in nodular black spots was completely infiltrated by inflammatory cells whereas in hyaline plaques the inflammatory cell infiltrate was limited to the outer borders of the lesions. The oval gaps characteristic of pleural plaques are absent in black spots.

Structures resembling black spots can be discerned in close proximity to overt mesotheliomas frequently diagnosed in former coal miners. The dark coal dust incorporations of the original visceral pleura represent a thin border line between the lung tissue and the greyish-white tissue of the malignant mesothelioma (fig. 4). In light microscopy, sometimes pre-existing dark mixed dust incor-

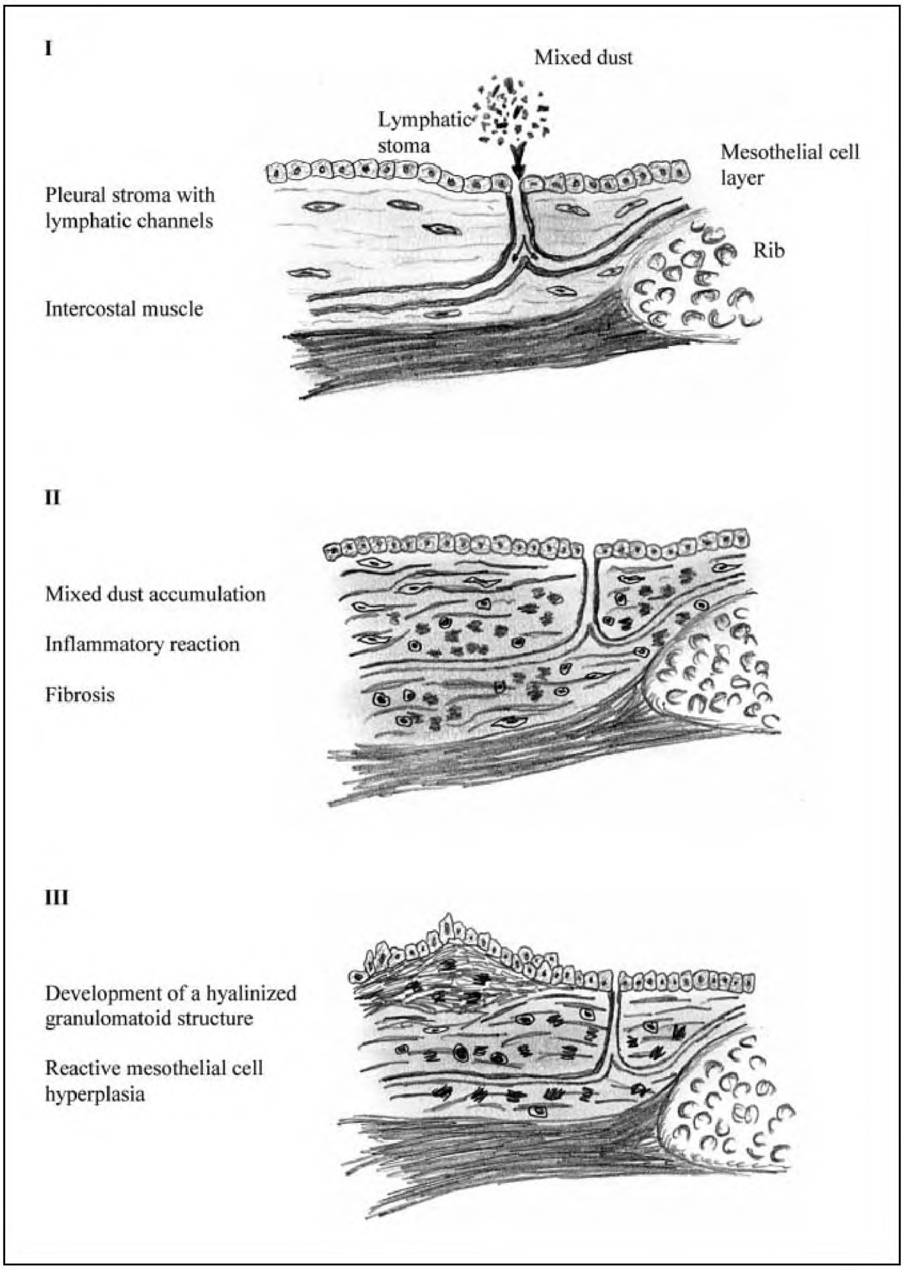

Fig. 3. Phasic development of pleural reaction within the regions of black spots.

porations are found adjacent to the fibrosed main layer of the origin parietal pleura together with an early development phase of an epitheloid mesothelioma (fig. 5).

Discussion

The parietal pleura covers the endothoracic fascia and borders on the intercostal musculature and the ribs. The surface facing the pleural cavity is covered by mesothelial cells whose diameter lies between 20 and 30 µm. The vasculature is embedded in the connective tissue layer of the pleura. In the costal region, the vascular supply to the parietal pleura is derived from branches of the intercostal arteries and intercostal branches of the internal thoracic artery. The vascular network overlying the ribs is much tighter compared to that overlying the intercostal muscles. The lymphatic stomata in the parietal pleura measure between 2 and 8 µm and are of particular importance as they represent a direct link between the pleural cavity and

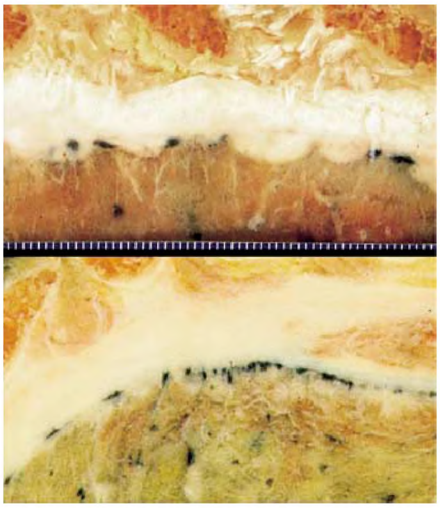

Fig. 4. Foci of dark mixed dust incorporations in segments of the original pulmonary pleura between lung tissue and the greyish-white tumor tissue of manifest mesothelioma.

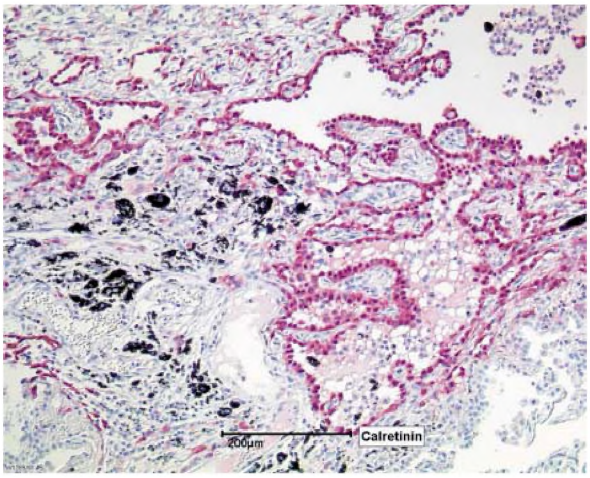

Fig. 5. Early development phase of an epithelioid mesothelioma. Pre-existing dark mixed dust incorporations adjacent to the fibrosed main layer of the origin parietal pleura (highly differentiated epithelioid mesothelioma stained with anticalretinin-antibodies).

the lymphatic system for the transport of fluid, fibrin and any corpuscular matter [6].

Black spots are dark patches resulting from carbon-rich mixed dust pigment incorporations in the main connective tissue layer of the parietal pleura. They are frequently found in the pleura of former coal miners. Following pigment deposition they continue to develop along pleural lymphatic channels.

According to the results of the energy dispersive X-ray microanalyses, the composition of the stored pigments depends on the occupational exposure to different types of mixed dust particles. Depending on the concentration of crystalline quartz, locally fibrosing reactions are variably pronounced progressing not uncommonly to the formation of typical, hyalinized granulomas. The reaction patterns to the incorporated minerals compare well with the pulmonary findings [7]. These reactive pleural lesions are basically benign.

Boutin et al. [5] described asbestos fibre accumulations within black spots. They concluded that black spots were predilection sites for the development of malignant mesotheliomas. Indeed, in areas of high concentrations of asbestos fibres in the lung or in the pleura an increased risk for development of a mesothelioma is given [8]. But due to our experiences the morphological finding of black spots is not an indicator for an existing mesothelioma or the possibility for the further development of a mesothelioma.

Our results do not support the hypothesis whereby mesotheliomas develop *preferentially* in the regions of pre-existing black spots. In our collective of former miners of the Ruhr area we do not find asbestos fibres especially amphibole fibres directly located in black spots. But we found silicates, quartz and silicone as well as aluminum-rich minerals such as muscovite. There were no hints that black spots play a possible role in the development of mesotheliomas

Irritation of mesothelial cells due to increased pigment incorporation and fibrotic alterations are not uncommon (fig. 4, 5) and have to be regarded as general pathological alterations, whereas mesotheliomas are caused by exposure to asbestos fibres and not by black spots which are generally free of asbestos fibres. In some cases, coexistence of these two independent lesions (black spots, mesothelioma) are seen. In cases of high exposure to asbestos fibres and resulting overload and altered lymphatic drainage, asbestos fibres might be found in black spots, too.

The occasionally increased proliferation tendencies of mesothelial cells overlying black spots in otherwise tumor-free tissue can at most be regarded as an indication for the classification of foci as possible sites of malignant tumor development. Up until now it was not possible to establish a firm link between so-called early mesotheliomas and pre-existing black spots. The fact that pigment incorporations like black spots are regularly and repeatedly identifiable within pulmonary pleural tissue without evidence of a co-existing malignant neoplasm on the lung surface is contradictory to the idea of obligate preneoplastic lesions as starting points in the development of malignant mesotheliomas.

In connection with the development of malignant mesotheliomas, incorporations of mixed dust particles can at most serve as a marker to map out the course of lymphatic channels. The given physiological conditions allow the pleural transport mechanisms to also trap asbestos fibres in the same places under certain circumstances, such as an occupational asbestos exposure. Mixed dust accumulations and the potential development of mesotheliomas at a later stage have to be viewed in the first instance as independent occurrences. Principal aspects concerning the role of black spots in the development of malignant mesotheliomas can only be ascertained by continued observations after thoracoscopy has provided the primary diagnosis of black spots. This also applies to the histological confirmation of the primary diagnosis of black spots and the documented neoplastic transformation of mesothelial cells adjacent to these foci.

References

1 Baffy G, Strate L, Krinsky M: Image of the month. Gastroenterology 2000;118:252, 453.

2 Worth G, Schiller E: Die Pneumokoniosen. Berlin, Staufen Verlag, 1954, pp 182–183.

3 Zenker F: Über Staubinhalationskrankheiten der Lungen. Dtsch Arch Klin Med 1867;2:116–172.

4 Müller K-M: Pleura; in Remmele W (ed): Pathologie. Berlin, Springer, 1992, vol 3, pp 715–732.

5 Boutin C, Dumortier P, Rey F, Viallat JR, De Vuyst P: Black spots concentrate oncogenic asbestos fibers in the parietal pleura: Thoracoscopic and mineralogic study. Am J Respir Crit Care Med 1996;153:444–449.

6 Li J, Zhao Z, Zhou J, Yu S: A study of the three-dimensional organization of the human diaphragmatic lymphatic lacunae and lymphatic drainage units. Ann Anat 1996;178:537–544.

7 Könn G, Schejbal V, Oellig W-P: Pneumokoniosen; in Doerr, W, Seifert, G (eds): Spezielle pathologische Anatomie. Pathologie der Lunge. Berlin, Springer, 1983, vol 16/II, pp 647–807.

8 Neumann, N, Günther S, Müller K-M, Fischer M: Malignant mesothelioma – German mesothelioma register 1987–1999. Int Arch Occup Environ Health 2001;74:383–395.

9 Rösler, H: Lehrbuch der Mineralogie. Leipzig, VEB, Deutscher Verlag für Grundstoffindustrie, 1979.