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CASE REPORT

Bilateral pleural effusion due to maligant mesothelioman, diagnosed by means of immunostaining

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Bilateral pleural effusion due to maligant mesothelioma, diagnosed by means of immunostaining. G.P.M. Mannes, A.S.H. Gouw, P.E. Postmus.
ABSTRACT: We report a patient who presented himself with a bilateral pleural effusion. Histology proved that this was caused by a malignant pleural mesothelioma. Immunostaining and DNA-flow cytometry confirmed the diagnosis. The usefulness of these rather new diagnostic techniques is discussed.
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Establishing an early diagnosis in patients with a malignant mesothelioma of the pleura might be rather difficult. New techniques such as immunostaining and DNA-flow cytometry can be of help, as we show in this case report.

Case report

A 46 yr old Asian male complained of nonproductive cough and dyspnoea during exercise for four weeks. Except for a cervical laminectomy two years earlier he had been in good health. After 20 pack-years, he stopped smoking 10 years ago. For about one year he had felt a vague and slowly progressive pain in his left hemithorax, without relation to breathing or exertion, and maximal when lying on his left side. Despite good appetite, he had lost 5 kg in weight. He used to work as a welder from about 25 years ago. Thirty years ago he had been working for a few months on a shipyard.

Physical examination revealed a healthy, slender man, with a normal blood pressure and central venous pressure. There were no enlarged lymph nodes. Heart sounds were normal. Over the basal part of the left lung, percussion revealed dullness and no breath sounds were heard. The liver was just palpable. Routine laboratory investigations showed no abnormalities. The purified protein derivative (PPD) was 20 mm. Rheumatoid factor and antinuclear antibody (ANA) were negative.

The chest roentgenogram (fig. 1) showed a bilateral pleural effusion, left more than right, and a broadened mediastinum. A computer tomography of the chest (fig. 2) showed the same pleural effusion, without evident pleural abnormalities. In the lungs, only a bulla in the right top was present. The mediastinum was broadened with an increased density, with probably some impression on the oesophagus.

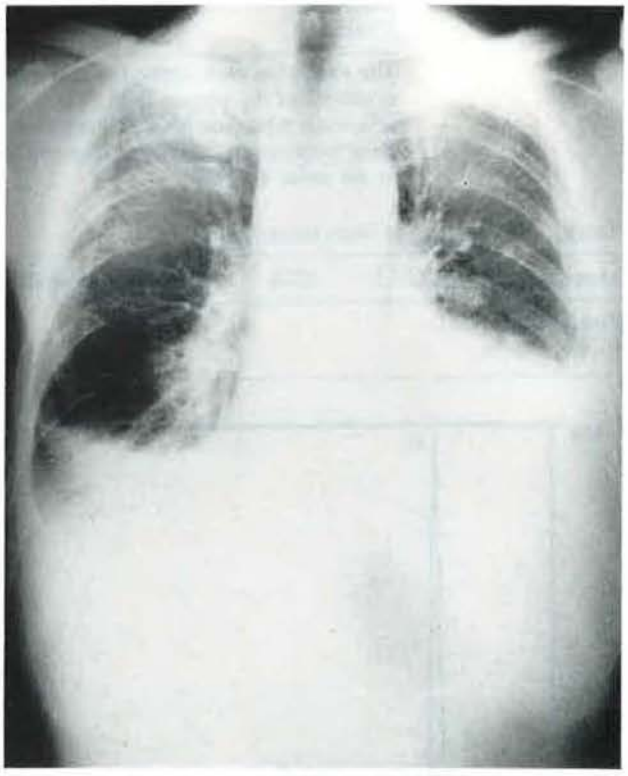

Fig. 1. – Chest-roentgenogram: bilateral pleural effusion.

Thoracocentesis on the left revealed a clotted blood stained fluid; 37 $g \cdot l^{-1}$ protein and lactate dehydrogenase (LDH) 1,155 $IU \cdot l^{-1}$. No acid fast bacilli were found. Cytological examination of the pleural fluid showed a hypercellular specimen in which, apart from inflammatory cells, individual and papillary clusters of cells were observed, with obvious nuclear enlargement and pleomorphism, atypical nucleoli and cytoplasmic

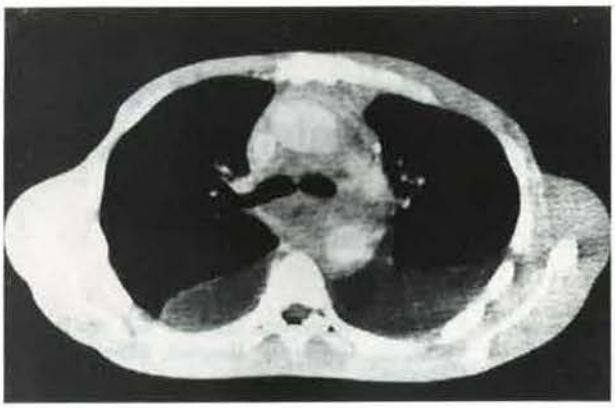

Fig. 2. – Computed tomographic scan of the thorax: bilateral pleural effusion and a broadened mediastinum.

vacuolization. These morphological features were consistent with malignancy. On discriminating between the possibilities, immunostaining proved to be useful as has been reported in other studies [1–7]. A panel of antibodies was applied, in our case, in an indirect immunoperoxidase technique (table 1). The malignant cells showed a staining pattern consistent with their mesothelial character and not with an epithelial differentiation: MOC-31 and CEA negative, RGE-53 and vimentin positive [4, 7].

At thoracoscopy on the left, 2.8 *l* of blood-stained fluid was evacuated. There were white and slightly elevated spots on the diaphragm, pleura parietalis and mediastinalis and on the lung. Three biopsies were taken from the spots on the pleura mediastinalis and showed reactive changes with fibrosis without evident malignancy. At thoracoscopy on the right, 2.2 *l* of fluid was evacuated. On the diaphragm a white and solid spot was seen, and smaller spots on the pleura parietalis. Biopsies were taken from both sites. The cytological examination of these samples revealed similar morphological and immunocytological features to the samples of pleural fluid from the left pleural cavity.

Table 1. – Specifities and sources of the antibodies

Antibody	Specificity	Source
MOC-31	epithelial cells [4]	Eurodiagnostics, NL
CEA	carcinoembryonic antigen	Dakopatts, Copenhagen, DK
RGE-53	keratin 18	Eurodiagnostics, NL
Vimentin	vimentin	Eurodiagnostics, NL

The antibodies were applied in a two-step immunoperoxidase technique by incubating the cytospins of the pleural fluid, which were fixed by acetone (10 min), for 30 min in the following dilutions: MOC-31 undiluted; CEA 1:200; RGE-53 1:20; vimentin 1:40. The same technique was performed on the biopsy material using snap frozen tissue specimens in the same dilutions.

Table 2. – Results of immunostaining

Material	MOC-31	CEA	RGE-53	Vimentin
Pleural fluid	-	-	+	+
Biopsy	-	-	+	+

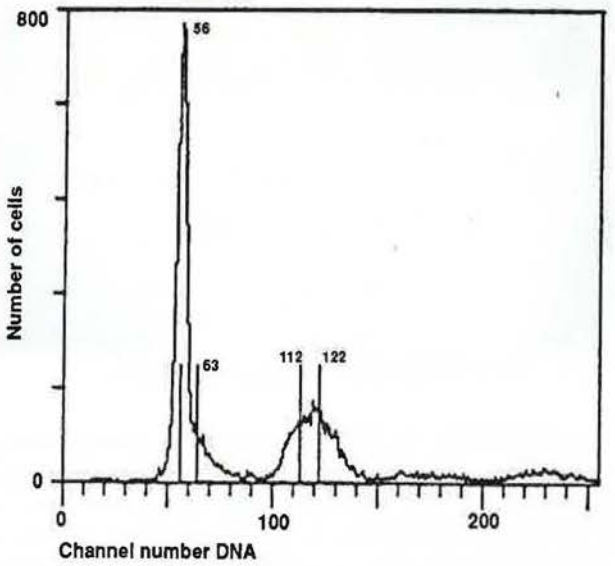

Fig. 3. – DNA-histogram of the cells harvested from the effusion of the left pleural cavity, showing an aneuploid cell population. The first peak is the reference peak, representing the normal cell population. DNA-index: 2.18. DNA: deoxyribonucleic acid.

DNA-flow cytometry of both fluid samples confirmed the malignant character of the mesothelial cell population, diagnosed earlier on morphological grounds, by the presence of the aneuploid peak in both samples (fig. 3) [8–10].

Microscopical examination of the biopsies of the right pleura parietalis and diaphragm showed solid groups and strands of tumour cells infiltrating fibrous tissue. The tumour cells showed obvious nuclear enlargement and pleomorphism, hypochromasia, atypical nucleoli and enhanced nuclear-cytoplasmic ratio. Immunohistologically, the tumour cells showed a pattern consistent with mesothelial and not with epithelial cells, similar to the results of the immunocytology: MOC-31 and CEA negative, RGE-53 and vimentin positive (table 2).

These findings lead to the diagnosis: malignant mesothelioma of the pleura (epithelial type), probably with mediastinal involvement.

Discussion

The first cases of malignant mesothelioma were published only 40 yrs ago. Although the incidence is increasing, it is still a relatively rare disease.

Many aetiological factors have been mentioned, but previous exposure to asbestos is by far the most important one. There is no clear dose-response relationship [11]. Although diminishing, asbestos is still used in many products these days, so almost everybody is exposed to it and at risk of the development of mesothelioma [12]. Our patient had had direct contact with asbestos thirty years ago, when he used asbestos in a shipyard.

Other possible aetiological factors are radiation therapy and exposure to vulcanic fibres (zeolites). Cigarette smoking does not appear to be of importance in malignant mesothelioma [13, 14]. Patients usually present with dyspnoea, chest pain or cough, as did our patient [13–15].

A unilateral pleural effusion is a common finding on a chest roentgenogram, but is also seen in patients with benign asbestosis. Bilateral pleural effusions are uncommon, especially at presentation. As the tumour extends, surrounding organs become involved such as the diaphragm, lung, pericardium, heart, chest wall, mediastinal structures and the contra-lateral pleura. In the majority of cases, lymphogenous and haematogenous metastases occur at a later stage of the disease and are found at autopsies in about 50%. Most of them are silent [13,15].

Diagnosis might be rather difficult. A combination of bronchoscopy, pleural fluid cytology and pleural needle biopsy is only diagnostic in about 60% [13,15,16]. Newer techniques, such as immunostaining (immunocytology and immunohistology) using a panel of antibodies and more recently DNA-flow cytometry, as applied in the present case, should raise this percentage.

Immunostaining is a useful aid to differentiate between mesothelioma and carcinoma. Differentiation on morphological features between mesothelioma and adenocarcinoma of whatever origin is a well-known dilemma. Absence of staining with MOC-31 and CEA, together with positivity for both RGE-53 and vimentin is a staining pattern consistent with mesothelial cells and not with epithelial (carcinoma) cells such as adenocarcinoma [2, 5, 7].

Another technique is DNA measurement by flow cytometry. In contrast to normal tissues, neoplastic lesions often undergo chromosomal aberrations resulting in the appearance of nondiploid (aneuploid) clones within the tumour cell population. Nondiploid patterns can be either unimodel, with one major cell population having an abnormal DNA content, or multimodal, with several distinct populations differing in their DNA contents. One of the fundamental issues of flow cytometry is the use of appropriate controls.

The DNA content in a cell population is presented as the DNA index. This is a formula used to express the position of histogram peaks in reference to the position of the normal diploid peak, usually determined by control measurements on normal lymphocytes or benign tissue of the same origin as the tumour [17].

Thus the aneuploidy by DNA-flow cytometry gives additional evidence to the morphology of malignancy, since reactive mesothelial cells are known to show atypical features too, but no aneuploidy [10].

Mostly, diagnosis is established by thoracoscopy and occasionally thoracotomy. There is no curative therapy, although there are some reports about the beneficial effects of chemotherapy. Occasionally an untreated patient survived more than 5 yrs [18].

The patient described here died at home, 6 wks after the diagnosis was made.

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Observation clinique. Epanchement pleural bilatéral chez un patient atteint de mésothéliome malin. G.P.M. Mannes, A.S.H. Gouw, P.E. Postmus.
RÉSUMÉ: Observation d'un patient atteint d'un épanchement pleural bilatéral. L'examen histologique montre qu'il s'agissait d'un mésothéliome pleural malin. Le diagnostic a été confirmé par un examen immuno-histologique et par la cytométrie de flux de la DNA. L'utilité de ces techniques de diagnostic relativement récentes est discutée.
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