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Simultaneous detection of tumour cells and positive genetic test results for *Mycobacterium tuberculosis* in pleural effusion

Abstract

Pleural effusion is a frequently observed lesion in the course of respiratory diseases such as inflammatory process and cancer metastasis. Its cause may be either tuberculosis (the most common extrapulmonary location is the pleura) and malignant disease of the pleura. Confirmation of tuberculosis is often troublesome. The primary site of cancer may be als difficult to find despite the application of difficult diagnostic methods. Below we present history of 79-year old female in whom carcinomatous cells and positive result of PCR for *Mycobacterium tuberculosis* in pleural fluid were discovered simultaneously suggesting the *tuberculosis* and cancer of unknown primary origin.

Key words: pleural *tuberculosis*, cancer of unknown primary site, immunohistochemical examination

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Introduction

Pleural effusion is a quite commonly observed abnormality in the course of many respiratory tract diseases, both of inflammatory and of malignant aetiology. Below we present a case of bilateral pleural effusion in which we detected genetic material of *Mycobacterium tuberculosis* and tumour cells. Despite extensive diagnostic evaluation we were unable to identify the primary.

Case presentation

A 79-year-old female managed for hypothyroidism, stable ischaemic heart disease, and hypercholesterolaemia, diagnosed with a diaphragmatic hiatal hernia, cholelithiasis, and degenerative changes in the spine, with a history of surgery for haemorrhoids, was admitted to our department due to dyspnoea and cough with purulent sputum expectoration worsening over the preceding five weeks. The patient had lost 3 kg during the 3 months preceding hospitalisation. She had never smoked and worked as a legal counsel. She had no family history of cancer. Physical examination revealed enlarged left supraclavicular and right axillary lymph nodes measuring up to 1.5 cm in diameter. They were soft, non-tender, and movable. There was a dull percussion tone and absent vesicular breath sounds below the scapular angles. Laboratory tests revealed the following findings: mild anaemia (HGB 11.92 g/dL, HCT 35.32%, RBC 3.87 × × 10^{12}/L), elevated thyroid stimulating hormone (TSH) to 7.3 μIU/mL (normal range: 0.27–4.20 μIU/ /mL) with normal free thyroxin (fT_4), elevated D-

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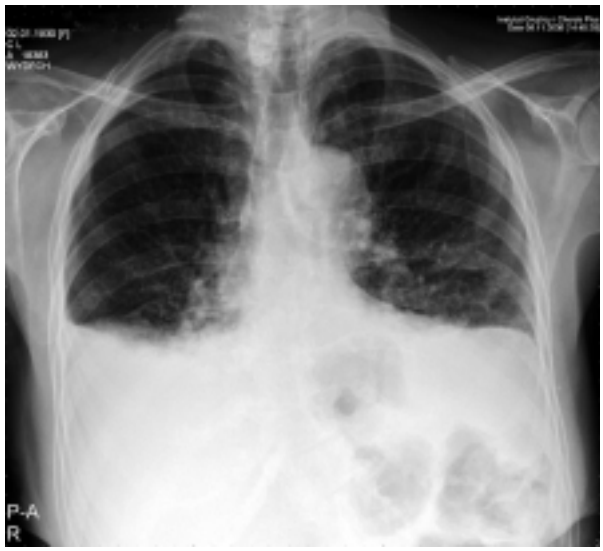

Figure 1. Chest X-ray — fluid in both pleural cavities, the right side extending to a height VIII ribs, on the left side to the IX rib and bilateral parenchymal density above the liquid level

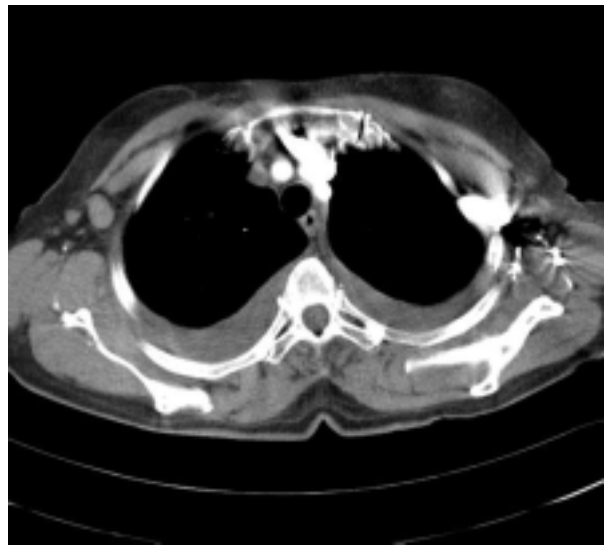

Figure 2. Patient's chest CT scanning revealing enlarged axillary lymph nodes

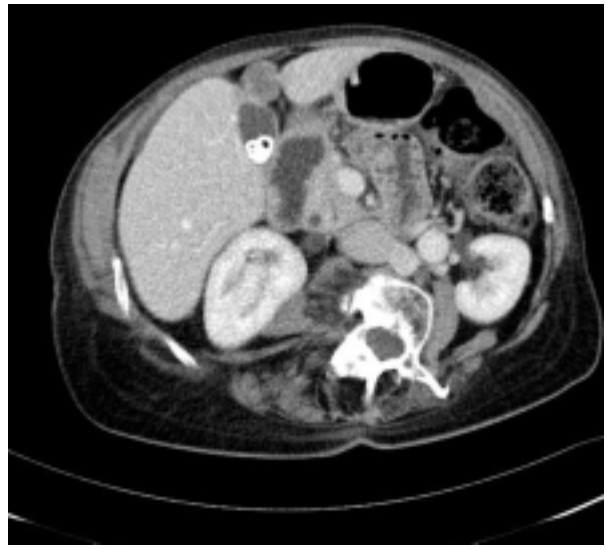

Figure 3. Patient's chest CT scanning revealing abnormal changes at the gallbladder

dimers to 1996 μg/L (normal range: 68–494 μg/L), and normal lactate dehydrogenase (LDH) activity of 604 U/L (normal range: 313–618 U/L). Arterial blood gas analysis was normal. A chest X-ray revealed bilateral pleural effusion reaching the eighth rib on the right and the ninth rib on the left and bilateral areas of parenchymal consolidation above the level of effusion (Fig. 1).

Several thoracocenteses were performed and a total of about 2000 ml of yellow fluid exudate showing characteristics of an exudate were evacuated. The microbiological evaluation of the exudate revealed genetic material of *Mycobacterium tuberculosis*, and cytology revealed papillary clusters of malignant cells. Pleural biopsy with an Abrams needle also revealed genetic material of *Mycobacterium tuberculosis* and histopathological evaluation demonstrated an adenocarcinomatous infiltrate. The fluid collected during another thoracocentesis revealed, again, fragments of *Mycobacterium tuberculosis* genome.

A chest CT scan revealed a normal pulmonary parenchyma except for small cicatricial lesions in the apices of the lungs. No enlargement of the hilar or mediastinal lymph nodes was demonstrated, although the axillary lymph nodes were enlarged to 18 mm (Fig. 2) and there was an 18–mm thyroid nodule with calcifications in the right lobe of the normal-sized thyroid gland. Flexible bronchoscopy revealed scars secondary to nodal punctures. The microbiological evaluation of the fluid obtained during bronchoalveolar lavage (BAL) for *Mycobacterium tuberculosis* (smears, genetic testing, culture) was negative.

In an attempt to identify the primary focus of the adenocarcinoma, numerous investigations were performed. An abdominal ultrasound scan revealed two round structures in the epigastrium, in the vicinity of the gallbladder: one measuring 26 × 18 mm and the other, located nearby, measuring 12 × 11 mm. In order to perform a more precise evaluation of these lesions a CT scan of the abdomen and pelvis was performed, which revealed a lesion near the gallbladder measuring 22 × 26 mm, of indeterminate nature (Fig. 3). A suspicion of a thickening of the stomach wall in the subcardial region was raised (Fig. 4). The right lobe of the liver revealed a non-specific hypodense focus measuring about 15 × 7 mm (Fig. 5) and enlarged inguinal

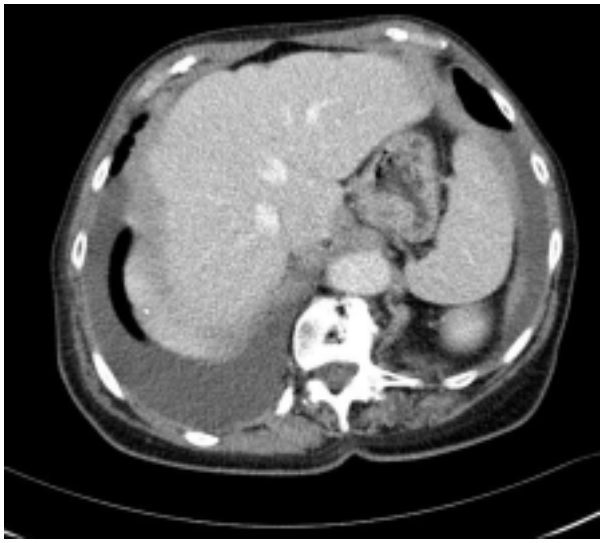

Figure 4. Patient's CT scanning revealing thickening of the stomach wall

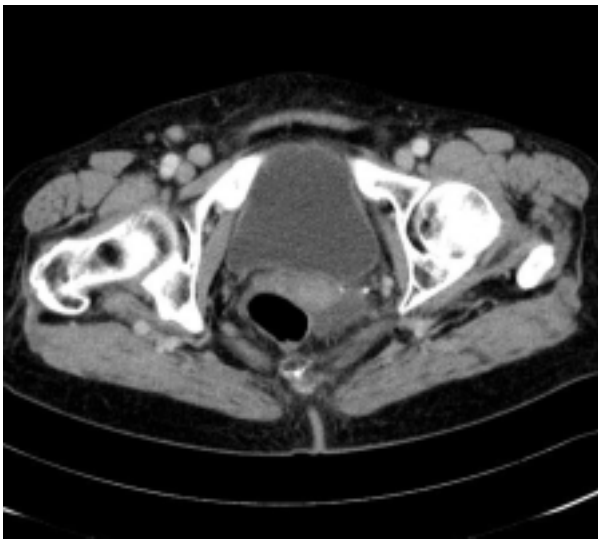

Figure 6. Patient's CT scanning revealing enlarged inguinal lymph nodes

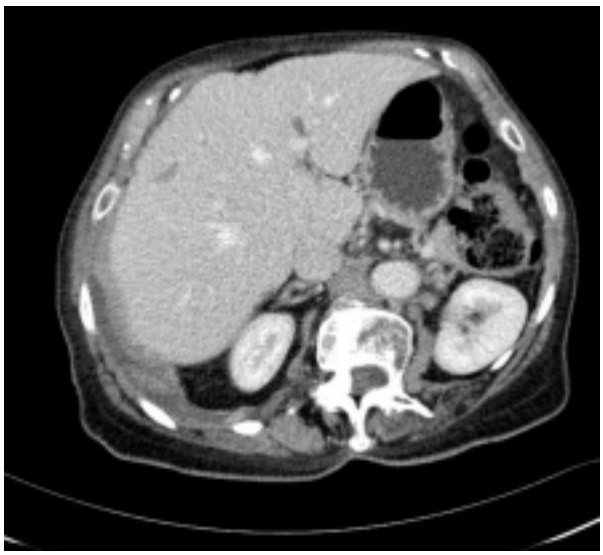

Figure 5. Patient's CT scanning revealing abnormal focus in the right lobe of liver

lymph nodes on the right side (Fig. 6). The remaining abdominal organs appeared normal.

Upper gastrointestinal endoscopy only confirmed the presence of a sliding hiatal hernia.

An ultrasound scan revealed several enlarged supraclavicular and cervical lymph nodes (with the largest one measuring 15 × 15 × 20 mm) which were hypoechoic and showed an altered structure. A biopsy of the left supraclavicular lymph node revealed non-small cell lung cancer cells. The pleural fluid and serum revealed high levels of CA 125 (serum: 423 U/mL [normal range: 0–35 U/mL], pleural fluid: 534 U/mL) and slightly elevated levels of cytokeratin 19 fragment (CYFRA 21-1) to 4.1 ng/mL (normal range: 0.3–3.0 ng/mL). The levels of carcinoembryonic antigen (CEA), CA 19-9, CA 15-3, and of neuron specific enolase (NSE) were all normal. The tissue samples collected during a closed pleural biopsy were stained for lung cancer and thyroid cancer (TTF-1), pleural mesothelioma (calretinin and HBME1, D2-40), adenocarcinoma (BerEp4), gastrointestinal adenocarcinoma (CEA, CK 20), and squamous cell carcinoma (p63) yielding negative results, while staining for upper gastrointestinal tract, pancreatic, and biliary tract cancer (CK7, CK19) was positive. Staining for breast cancer was negative for the progesterone receptor (PgR) and positive in some cells for the oestrogen receptor (ER). Immunohistochemical (IHC) staining supported metastatic breast or ovarian cancer and were less typical of pancreatic or biliary tract tumours.

The diagnosis of cancer (adenocarcinoma) was made involving the pleura and supraclavicular lymph nodes of unknown primary in a patient with positive results of a polymerase chain reaction (PCR) for *Mycobacterium tuberculosis*. The disseminated cancer and the advanced age rendered the patient ineligible for systemic cytotoxic treatment. Due to the positive genetic tests for acid-fast bacilli on three separate occasions, the patient was started on a standard anti-tuberculous regimen consisting of isoniazid, rifampicin, and pyrazinamide. The initial tolerance was good, but after two weeks of treatment the patient developed nausea and upper right quadrant pain accompanied by elevated AST to 147 IU/L (normal range: 14–36 U/L). The anti-tuberculous drugs were discontinued, and in order to continue treatment the patient was transferred to the Mazovian Centre for the Treatment of Tuberculosis and Lung Diseases in

Otwock. The three sputum cultures and pleural fluid cultures on egg media were negative for acid-fast bacilli at ten weeks.

During the hospitalisation at the Mazovian Centre for the Treatment of Tuberculosis and Lung Diseases, the anti-tuberculous treatment was continued, but due to the increasing amount of fluid in both pleural cavities regular bilateral thoracocenteses were performed every 10–14 days. It was decided not to perform pleurodesis, to prevent the increase in the amount of the pleural fluid in both pleural cavities. After the hospitalisation at the Mazovian Centre for the Treatment of Tuberculosis and Lung Diseases the patient continued anti-tuberculous treatment at home. In spite of this, no reduction in the amount of fluid in the pleural cavities could be achieved, which might have suggested that the primary cause of the pleural exudate was cancer rather than, as initially believed, pleural tuberculosis.

Discussion

Tuberculosis (TB) is an infectious disease caused by acid-fast bacilli from the *Mycobacterium tuberculosis* group. About a third of the world population is believed to be infected with *Mycobacterium tuberculosis*. The pleura is the most common extrapulmonary site of tuberculosis (40.7% of cases of extrapulmonary tuberculosis) [1, 2]. In Poland, patients with extrapulmonary tuberculosis accounted for 7.4% of the total number of patients with tuberculosis registered in 2008 [1, 2]. The extrapulmonary location of tuberculosis poses a considerable diagnostic problem. In the case of pleural tuberculosis a definite confirmation of the diagnosis is made possible by positive cultures of pleural fluid or of tissue samples obtained during a pleural biopsy. Due to the fact that the pleural fluid contains few mycobacteria, direct microscopy is positive in a little less than 15% of cases, while positive cultures are only observed in 12–70% of the patients [1–3]. In the case of our patient the decision to start anti-tuberculous treatment was not an easy one to make. The diagnosis of tuberculosis was supported by three results of the molecular investigations, confirming the presence of the genetic material of *Mycobacterium tuberculosis* in the pleural fluid and a pleural tissue sample. Given that no *Mycobacterium tuberculosis* was cultured in the sputum or the pleural fluid, we considered the possibility of false positive results of the genetic testing by PCR, which might have been caused, for instance, by cross-contamination of the samples. The genetic methods, due to possible "over-detectability", do not allow a definite diagnosis of tuberculosis to be made [3–6]. In the case of bacterioscopy-negative samples, genetic methods have a sensitivity of 22–81% and specificity of up to 100%, which allows them to be used as ancillary tests for the diagnosis of tuberculosis, particularly tuberculosis cases with few mycobacteria, in which it is difficult to obtain positive cultures [1–3]. Anti-tuberculous treatment can also be justified in cases in which the diagnosis of tuberculosis is likely or just possible, namely in bacteriologically unconfirmed cases. Another fact suggestive of active tuberculosis which justified the initiation of anti-tuberculous treatment in our patient was the presence of signs pointing to a history of tuberculosis: small cicatricial lesions in the pulmonary apices on chest CT and scars in the bronchi secondary to nodal punctures on bronchoscopy. Furthermore, according to the literature, cancer increases the risk of reactivation of tuberculosis [3]. By administering anti-tuberculous treatment we hoped to reduce the pleural effusion that our patient found so troublesome. We also feared that the tuberculous process would disseminate, given the presence of cancer. There was also a possibility of an adverse impact of tuberculosis on the course of the patient's cancer [3, 4].

Another diagnostic problem was the detection of cancer cells and the impossibility of establishing the primary focus of the cancer. Cancer of unknown primary (CUP) accounts for about 2–5% of all cancers, with some authors reporting rates of up to 10% [6–7]. The most common CUPs include: low- to intermediate-grade adenocarcinoma (about 60%), high-grade adenocarcinoma (about 30%), squamous-cell carcinoma (about 5%), undifferentiated cancer (about 5%), and, rarely, cancer with neuroendocrine features [7–9].

The currently used additional staining methods and other pathological diagnostic methods increase the likelihood of establishing the primary origin in a further 10% of cases initially diagnosed as CUPs [8, 10]. Our patient was in the remaining 90% of such cases, in whom the primary could not be identified in spite of imaging, pathologic, immunohistochemical, and biochemical diagnostics [9–12].

Imaging studies play an important role in the diagnostic evaluation of cancer. As McMillan et al. have demonstrated, abdominal CT is far more precise than abdominal ultrasound [11]. Abbruzzese et al. managed to establish the primary focus in 20% of 879 patients with CUP using CT imaging [12]. Currently, PET-CT (positron emission tomo-

graphy-computed tomography) is a very helpful diagnostic tool. Rusthoven et al. have shown that by using PET-CT it is possible to identify the primary in 25% of patients with CUP and that additional metastatic foci can be found in 27% of examined patients. The study was conducted on patients with cervical lymph node involvement [13]. It is believed that PET-CT enables identification of the primary in 8–53% of patients. Nevertheless, one should bear in mind that false positive results may be obtained in nearly 20% of patients, mainly due to the presence of inflammatory foci [14]. MRI imaging of the breast is a useful tool in the diagnostic evaluation of axillary lymph node enlargement in patients with suspected breast cancer, particularly in those with normal mammograms [15].

Our patient underwent mammography several months prior to hospitalisation. The mammography was performed at a centre in which she had had it done for the past few years. As the mammogram was normal and the IHC staining of the biopsy material did not unequivocally suggest breast cancer, no MRI imaging was ordered. Additionally, determinations of cancer markers added little to the diagnosis. While there were elevations in CA-125 levels (a helpful marker in the evaluation of ovarian cancer) both in the pleural fluid and the serum, these findings may have been caused by the sole presence of exudate in body cavities. What is more, elevated CA-125 can be observed in the course of tuberculosis [16, 17]. Markers determined in the evaluation of cancer (CA 15-3, CA 19-9, CA-125, CEA) are non-specific and do not enable unequivocal diagnosis of a specific type of cancer, as was the case with our patient [7, 16, 18]. IHC staining of tissue samples seems very helpful [7, 14, 16]. The IHC staining suggested breast or ovarian cancer and was less typical of pancreatic or biliary tract cancer. Abdominal imaging did not reveal any abnormalities within the pancreas, and the ovaries were not visualised, which suggested other organs as the site of the primary focus. According to various investigators, the combination of positive staining for cytokeratin 7 and negative staining for cytokeratin 20 may be suggestive of breast, ovarian, lung, endometrial, bile tract, or pancreatic cancer [19, 20]. Despite the normal mammography, which was performed in an outpatient setting, breast cancer cannot be conclusively ruled out. However, the advancement of the cancer and the IHC staining results suggestive of a low likelihood of response to anti-oestrogen therapy prompted us to initiate supportive care and not to continue further evaluation that would include MRI imaging of the breasts.

The clinical picture of patients with CUP varies and depends on the location of metastases. The primary is often too small to be located using the available diagnostic methods. At diagnosis the patient's performance status was good (PS 2), she did not require third party assistance, and her activity was limited by the dyspnoea secondary to pleural effusion. The detection of mycobacterial genetic material obliged us to initiate anti-tuberculous treatment, and the identification of tumour cells in the pleura and supraclavicular lymph nodes made successful anti-tumour treatment impossible given the numerous co-morbidities. The necessity of pleural fluid evacuation every 10–14 days suggests that the main cause of the pleural effusion was cancer. It seems that pleural obliteration using, for instance, talc or bleomycin pleurodesis would be beneficial at this stage of the disease [21, 22].

Conclusions

The differential diagnosis of pleural effusion can be a serious problem. Despite using state-of-the-art immunohistochemical and microbiological methods (including those based on genetic testing), both the evaluation of tuberculosis and cancer can be inconclusive.

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