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CASE REPORT

High amylase activity in pleural fluid and primary bronchogenic adenocarcinoma

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High amylase activity in pleural fluid and primary bronchogenic adenocarcinoma. O. Devuyst, M. Lambert, J.M. Scheiff, J. Francart.
ABSTRACT: We report a case of primary bronchogenic adenocarcinoma, complicated by pleural effusion, in which very high pleural amylase activity was found, whilst serum amylase was normal. Isoamylase determination showed a salivary-type amylase. Concerning the origin of this enzyme, ultrastructural study of the malignant cells obtained from the pleural fluid suggested a local amylase synthesis. The pathophysiological significance of electron-dense granules found in these cells is also discussed. *Eur Respir J., 1990, 3, 1217–1220.*

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It is well known that a high number of non-pancreatic and non-salivary disorders are associated with hyperamylasaemia, especially several non-neoplastic lung diseases but also bronchogenic and ovarian carcinoma [1, 2].

Ectopic amylase production by a lung cancer was first suggested by Weiss *et al.* [3] in 1951. Since then, many authors have reported similar cases, but there has only been one case reported where increased amylase activity was restricted to the pleural fluid [4]. Biochemical studies of both serum and pleural amylase have revealed a marked proportion of the salivary (S-type) isoenzyme, with some peculiar variants [5]. In lung carcinoma, ultrastructural studies have disclosed the presence of electron-dense granules in the tumour cells, which were considered to be zymogen granules [6].

We report the case of a patient with a primary bronchogenic adenocarcinoma associated with a marked elevation of pleural amylase activity but without hyperamylasaemia. We also describe the electronmicroscopical characteristics of the pleural tumour cells, which were compatible with local amylase synthesis.

Case report

A 53 yr old woman was admitted to hospital in March, 1988, because of right-sided thoracic pain, cough and progressive dyspnoea of two month-duration. She was a nonsmoker and her past history was irrelevant. On admission, physical examination showed only a right-sided pleural effusion. There was no lymphadenopathy. Chest X-ray confirmed the right-sided pleural effusion, but did not reveal any tumour.

Laboratory tests were within normal limits, except for a mild thrombocytosis (429,000 cells·mm^{-3}). Serum and urine amylase values were normal. Examination of the pleural fluid revealed: a high level of protein (54 g·l^{-1}), lactate dehydrogenase (LDH) (380 IU·l^{-1}) and amylase (1,004 IU·l^{-1}); a raised level of carcinoembryonic antigen (CEA)(8 ng·ml^{-1}) while serum CEA was normal (1.5 ng·ml^{-1}, normal values: 0–3 ng·ml^{-1}); the presence of malignant cells on cytological examination and negative microbiological studies.

After removal of about 1,000 ml of pleural fluid, control chest X-ray and tomography disclosed a round solitary nodule (18 mm in diameter) in the right middle lobe. Fibreoptic bronchoscopy was normal and the cytological examination of the bronchial aspiration fluid remained negative. Thoracic computerized tomography (CT)-scan confirmed the presence of a spiculated nodular lesion in the internal segment of the middle lobe with mediastinal and right hilar lymphadenopathies. Extensive search for an extrapulmonary tumour, in particular mammary, pancreatic, digestive and ovarian carcinoma, remained negative.

We finally diagnosed a primary bronchogenic carcinoma, with pleural and lymph node metastases.

Chemotherapy with carboplatin-vindesine-methotrexate was given twice, at a one month interval, without any substantial benefit. The patient was then treated symptomatically by repeated pleural aspirations and local talc application. She died 11 months after the first presentation. The autopsy confirmed the diagnosis of primary bronchogenic tumour with major pleural involvement and peritoneal carcinomatosis.

Special studies

Amylase levels in the pleural fluid ranged from 833–1,598 IU·l^{-1} over an eight month period. Serum amylase was always within normal limits (20–200 IU·l^{-1}), except for the last two determinations, which showed slightly elevated values (215 and 239 IU·l^{-1}).

Isoenzyme determination was performed, according to O'DONNELL *et al.* [7], on a sample of pleural fluid, with a total amylase activity of 1,251 IU·l^{-1}: 83% of which was of the salivary type, whilst only 17% was of the pancreatic type.

Optical microscopic examination of the cell pellet obtained from the pleural fluid disclosed a mixed population of inflammatory elements and a moderate amount of tumour cells, ranging from small, undifferentiated cells to large, vacuolated ones. They were either dispersed as single elements or arranged in small clusters or glandular structures.

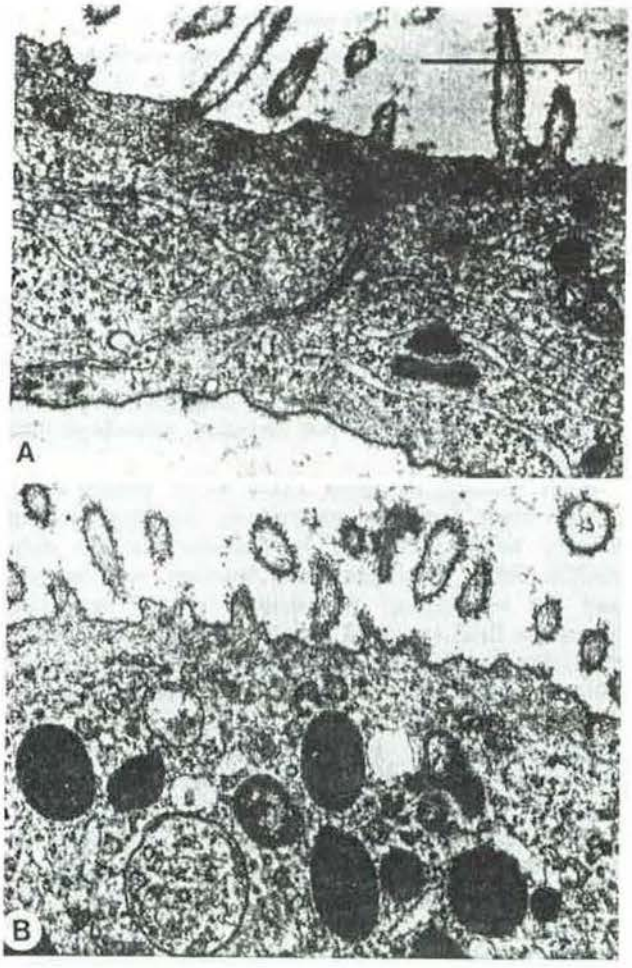

Fig. 1. – A: A junctional complex joining the cytoplasms of adjacent cells in a neoplastic gland; the anchoring system resembles those found in columnar epithelia. Note also the well developed cisternae of rough endoplasmic reticulum in both cytoplasms and the presence of some granules in the right one. (Bar represents 0.9 μm). B: Portion of the outer, "apical" cytoplasm of a tumour cell displaying numerous microvilli and containing big, electron-dense granules, most of which have a homogeneous appearance. (Scale as A.)

Ultrastructural examination revealed that the neoplastic cells in the aggregates and glands were closely apposed, at least in some areas, and connected by true anchoring systems, comprising tight and intermediate type junctions, as well as desmosome-like structures (fig. 1a). The cell margin was always covered with numerous microvilli (fig. 1b); in single cells, these were present on the whole cell surface; in aggregated cells, they developed only on the free surface, *i.e.* the area of the cells in contact with the pleural fluid (fig. 2). The polarity of the cells forming the wall of the glands was inverted, microvilli being present only on the outer membrane, while the inner, adluminal one, was smooth. In consequence, this characteristic gave the glands a peculiar inside-out appearance. The nucleus had an irregular outline, with more or less deep folds and notches. Chromatin was generally dispersed, giving it a pale appearance. The nucleoli were prominent and composed of a thick nucleolonema which exhibited focal condensations of its filamentous component. The broad cytoplasm contained many free polyribosomes, extensive cisternae of rough endoplasmic reticulum, fairly well developed Golgi complexes and more or less large-sized vacuoles. Numerous elongated mitochondria were a constant feature (fig. 2).

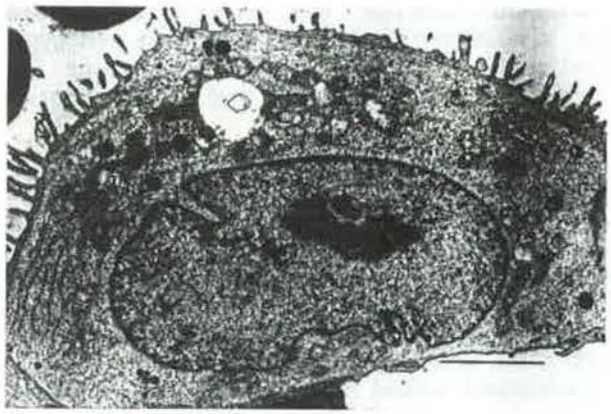

Fig. 2. – A tumour cell belonging to the wall of a free-floating glandular structure. Microvilli are present only on the outer membrane in contact with the pleural fluid. The nucleus is irregular and contains mainly euchromatin as well as a band-like nucleolus. The cytoplasm shows numerous mitochondria, some strands of rough endoplasmic reticulum and dispersed granules of different sizes. (Bar represents 2.6 μm).

Of particular interest was the presence of electron-dense granules in almost every tumour cell (fig. 1a and b; fig. 2). Their size ranged from very small bodies up to large granules, approximately 0.5 μm in diameter. Their shape was round to oval, sometimes irregular. They were always surrounded by a single limiting membrane, but their matrix showed a variable texture: the big round granules were usually homogeneous; the smaller irregular ones had a content varying in electron density and were sometimes distorted by cytoplasmic bulges. However, a lamellar or annulate pattern was never found. The granules were scattered in small numbers throughout the entire cytoplasm, with a somewhat higher numerical density in the "apical" region.

Unfortunately, the poor preservation of the autopsy material did not allow a conclusive comparative ultrastructural study between the pleural malignant cells and those of the primary tumour.

Discussion

There are few reports concerning pleural amylase content, other than in pancreatitis and oesophageal perforation [8, 9]. Among these cases, about 40% are associated with lung carcinoma (mainly adenocarcinoma), generally with pleural or mediastinal metastatic invasion and most frequently accompanied by hyperamylasaemia. Interestingly, all the isoamylase determinations revealed a predominant salivary (S-type) isoamylase, with some differences in the kinetic properties on electrophoresis.

The association reported here, of lung carcinoma and increased S-type pleural amylase activity, without concomitant hyperamylasaemia, is the most uncommon situation. Only one similar case has been reported in the English literature [4].

The origin of amylase in pleural fluid, associated or not with hyperamylasaemia, is of interest. The normal lung can produce amylase. In 1961, ENDE [10] described the presence of an "amylase-like" substance in the normal lung. BERK *et al.* [1] reported that this physiological amylase activity was predominantly of the salivary type. Recently HAYASHI *et al.* [11], using an immunohistochemical method, revealed the presence of amylase in the serous glandular cells and the ciliated epithelial cells of the bronchus. In the serous cells, the staining pattern was granular, suggesting that amylase was located in secretory granules. Since lung extracts from patients suffering from pulmonary infections, such as tuberculosis or pneumonia, have increased amylase levels, the physiological role of this enzyme might be antibacterial [5].

In the case of serum or pleural amylase associated with lung carcinoma, the most attractive explanation for their origin would be the synthesis of the enzyme by the tumour cells. A classic example of such a paraneoplastic secretion is the Regan isoenzyme of alkaline phosphatase [12]. The formation of enzymes by tumour cells could thus be compared with the well known secretion of peptide hormones by lung carcinoma. In this regard cases where lung carcinoma was associated not only with hyperamylasaemia but also with the production of adrenocorticotrophic hormone (ACTH) or melanocyte stimulating hormone (MSH), are particularly interesting. In these patients, direct proof of amylase production by neoplastic bronchogenic cells was furnished by histochemical and immunohistochemical methods [13]. Moreover, using a culture technique, UEDA *et al.* [14] demonstrated amylase production in isolated tumour cells of an ovarian adenocarcinoma.

In our case, the development of the rough endoplasmic reticulum and the Golgi complexes argue in favour of secretory protein synthesis, compatible with amylase production. Hitherto, however, the precise intracellular localization of this enzyme has not been determined. Several authors [4, 6, 13] have considered the dense granules found in the tumour cells as being the storage site. Indeed, the granules bear some resemblance to zymogen granules, and their preferential localization in the apical region of the cytoplasm recalls the distribution of secretory granules in exocrine cells. However, without further proof they cannot be considered to be a constant ultrastructural hallmark of amylase secreting adenocarcinoma.

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Hyperamylopleurie et adenochrcinome bronchique primitif. O. Devuyst, M. Lambert, J.M. Scheiff, J. Francart.
RÉSUMÉ: Nous rapportons un cas d'adénocarcinome bronchique primitif, compliqué d'un épanchement pleural, dans lequel une très importante activité amylasique a été décelée, bien que l'amylase sérique fût normale. L'étude des isoenzymes a révélé qu'il s'agissait d'une isoamylase de type salivaire. L'examen ultrastructural des cellules malignes de l'épanchement pleural suggère une synthèse locale d'amylase. La signification physiopathologique de granules denses dans ces cellules est également discutée.
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