

Neuroendocrine cells in anthracosilicotic lungs

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Neuroendocrine cells in anthracosilicotic lungs. H.L.L. Gubbelmans, B.L.P. Maesen, J. Koudstaal, F.B.J.M. Thunnissen.

ABSTRACT: In normal human lungs, neuroendocrine (NE) cells can be found as solitary NE cells and in corpuscular aggregates, the neuroepithelial bodies. By means of a quantitative morphometric method, we compared the number and distribution of chromogranin-reactive NE cells in anthracosilicotic and control lungs. After death, 14 pairs of control and 12 pairs of anthracosilicotic lungs were sampled.

The extents of anthracosilicosis and emphysema were described for the right lung. The left upper lobe was used for quantitative determination of the number and distribution of NE cells, visualized by immunohistochemical staining for chromogranin. In contrast to expectations, we found no statistical difference in the numbers of chromogranin-reactive NE cells per 10 cm of epithelium between control (11.0) and anthracosilicotic (2.4) lungs.

In both groups, there was a much greater number of chromogranin-reactive cells in the more peripheral compared to the central airways. Most peripheral NE cells were arranged in neuroepithelial bodies, which were not found in the central airways.

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In contrast to the gastrointestinal tract, neuroendocrine (NE) cells were discovered relatively late in human lungs [1] and even then only through their tumours (oatcell carcinoma and bronchial carcinoid tumour [2]). In the human bronchial epithelium NE cells are relatively sparse and they cannot be distinguished readily from other epithelial cells by ordinary haematoxylin-eosin staining. New histochemical and immunohistochemical techniques stain the contents of NE cells selectively [3] and have made it possible to recognize the NE cells easily among the other cells of the bronchial epithelium such as goblet cells, ciliated cells and basal cells. Some studies [4-6] have suggested that the numbers of NE cells are increased in bronchiectatic, emphysematous and carcinomatous lungs. However, none of these studies was designed in a quantitative way.

The purpose of the present study was to count the number of NE cells using morphometric techniques [7, 8] in anthracosilicotic lungs and to compare the density and distribution of these cells with control lungs.

In Heerlen, a former coal-mining area, we were able to sample 12 pairs of silicotic lungs in sections. Because there are extensive fibrotic changes in silicotic lungs [9] and some of these coal miners were known to have respiratory insufficiency, we expected, in parallel with the above-mentioned lung diseases, to find more NE cells in anthracosilicotic lungs.

Material and methods

From 26 patients who came to necropsy within 6 h after dying in the De Wever Hospital, Heerlen from 1988-1990, the lungs were removed from the thorax and fixed according to a standard protocol (see below). Twelve patients were former coal-miners and were known to have anthracosilicosis. Among these there were three with macular, five with nodular and four with pseudotumoral forms of anthracosilicosis. Five of the twelve specimens came from miners with well-established respiratory insufficiency. The lungs of fourteen patients were used as a control group. Excluded from the control group were lungs with tumours, emphysema, bronchiectasis and lungs of patients known to have respiratory insufficiency. All the anthracosilicotic lungs were from male subjects, and nine out of fourteen control pairs were from females. The ages of both groups were comparable: 70.8 ± 11.5 yrs (mean \pm SD) in the anthracosilicotics and 66.3 ± 18.7 yrs in the controls.

The lungs were fixed within 15 min after necropsy to prevent sloughing of the epithelium. The lungs, trachea and larynx together with the heart were carefully removed "en bloc" from the thorax according to a fixed protocol. The vessels of both hila were ligated and the heart was detached. Subsequently, a rubber tube was inserted proximal in the trachea and the lungs were distended for at least 24 h at a constant transpulmonary pressure of

3 kPa (30 cm of 4% formaldehyde solution) while they floated in a large container filled with 4% buffered formaldehyde [10, 11]. After fixation, the tube was removed, the trachea ligated and the lungs were preserved immersed in a container of formaldehyde.

The volume of both lungs was measured by immersion in a bucket filled with water. The right lung was cut in the sagittal plane from the hilum into slices with a thickness of 1 cm. These slices were used to describe the nature and extent of silicosis [12] and emphysema macroscopically. Three blocks of tissue were taken from each of the three lobes of the right lung and embedded in paraffin.

The left lung was cut according to the method of TATEISHI [13] (fig. 1). Blocks of tissue were taken from the left upper lobe bronchus to the inferior segment of the lingula after each bifurcation of the bronchus until seven sites had been sampled. All of these samples were embedded in paraffin and serial 4 μ m sections were cut. Consecutive sections were stained with haematoxylin-eosin and for chromogranin [14, 15] with the immunoperoxidase method.

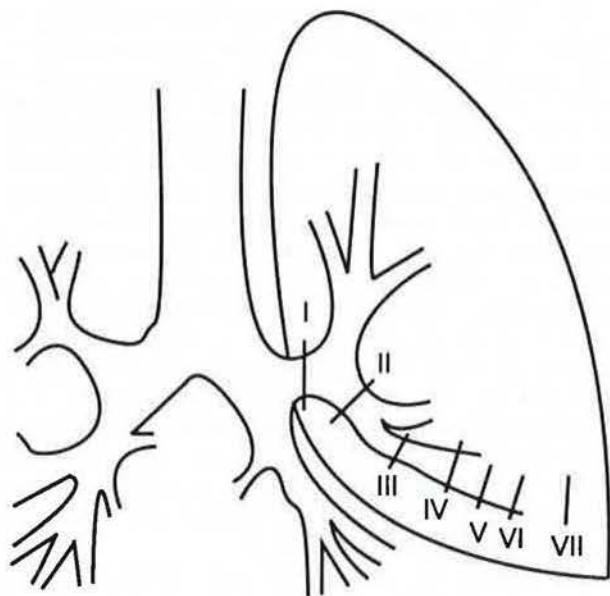


Fig. 1. - Sections of the left upper lobe from central (I) to the most peripheral airways (VII). Modification of the method of TATEISHI [13].

For the immunoperoxidase staining, the paraffin embedded sections were mounted, deparaffinized and rehydrated with tris buffer salt (TBS) solution. In order to reduce nonspecific binding of the antibody, the sections were incubated for 15 min in 1% bovine serum albumin/TBS. After removal of the serum the sections were incubated for 1 h with mouse anti-chromogranin (DAKO-ITK; diluted 1:10000). Then, after rinsing in TBS, the sections were incubated with RaM (rabbit anti-mouse) biotin (DAKO-ITK; diluted 1:300), rinsed for 30 min in TBS, incubated with streptavidine-conjugated peroxidase (DAKO-ITK; diluted 1:600) for 30 min,

rinsed in TBS and finally incubated with the chromogen containing diaminobenzidine (DAB) with 0.002% H_2O_2 . After rinsing in TBS the sections were counterstained with Harris' haematoxylin and mounted in malinol (Chroma).

The extents of emphysema and silicosis were microscopically identified on the haematoxylin-eosin stained sections of the right lung.

We then counted the number of chromogranin-reactive NE cells in the bronchial epithelium in each chromogranin-stained section of the left upper lobe. A neuroepithelial body was defined as a cluster of 3 or more NE cells. The length of the bronchial epithelium was measured using the following method. The bronchial and bronchiolar circumferences were calculated using the diameter (D) for the circular bronchioli (circumference = $3.14 \times D$), and using the short (d) and long (D) diameters (circumference = $3.14 \times d + 2 \times (D - d)$) for the more oval bronchi. Subsequently, the numbers of chromogranin-immunoreactive cells per 10 cm of bronchial epithelium were computed. In the anthracosilicotic group, the density of NE cells was compared between hypoxic (n=5) and nonhypoxic (n=7) coal-miners. For statistical analysis the Mann-Whitney U test was used.

Results

Microscopic examination of the haematoxylin-eosin stained slides from the anthracosilicotic group showed a considerable degree of anthracosilicosis in all specimens. These signs of anthracosilicosis were not present in the control group and confirmed the macroscopic findings.

In all of the anthracosilicotic lungs we found a considerable degree of emphysema, predominantly of the centrilobular type. The lengths of the bronchial epithelium measured in both groups were comparable, 111 cm for the anthracosilicotic group and 123.5 cm for the controls. However, there was a considerably higher number of chromogranin-reactive cells per 10 cm of epithelium in normal (11.0 ± 2.2 ; mean \pm SD) than in anthracosilicotic lungs (2.4 ± 3.7). This difference was not statistically significant ($p > 0.05$).

The numbers of chromogranin-reactive cells per 10 cm of bronchial epithelium in the hypoxic and nonhypoxic coal-miners were, respectively, 4.4 and 1.2. However, this difference was not statistically significant ($p > 0.05$).

In both anthracosilicotic and normal lungs, the numbers of solitary NE cells were larger than the numbers of neuroepithelial bodies; nevertheless the contribution of the neuroepithelial bodies to the mean number of chromogranin-reactive NE cells was greater, because neuroepithelial bodies contained a wide range (3-80) of NE cells. Comparison of the numbers of reactive cells between male and female lungs in the control group did not reveal a significant difference. Therefore, we considered the control group homogeneous.

The mean number of chromogranin-reactive NE cells was calculated for each section of the left upper lobe from I (central) to VII (peripheral airway) in control and anthracosilicotic lungs (fig. 2). In both groups there was

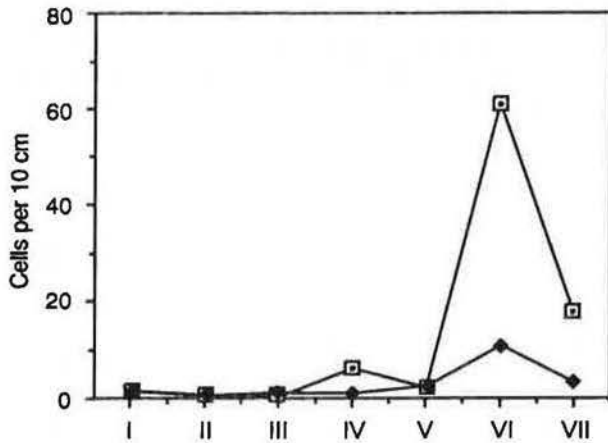


Fig. 2. - Densities of neuroendocrine cells from central (I) to the most peripheral airways (VII) in the left upper lobe for control and anthracosilicotic lungs. — □ — : controls; — ◆ — : anthracosilicotic.

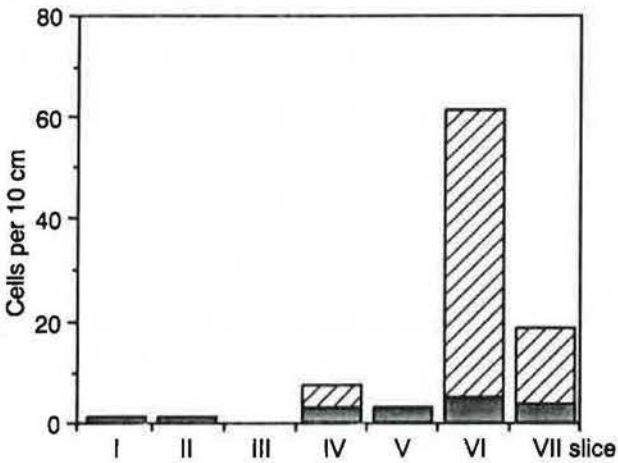


Fig. 3. - The distribution of the solitary neuroendocrine cells and neuroepithelial bodies within the mean number of neuroendocrine cells in consecutive slices of the left upper lobe in control lungs: ■ : solitary neuroendocrine cells; ▨ : neuroepithelial bodies.

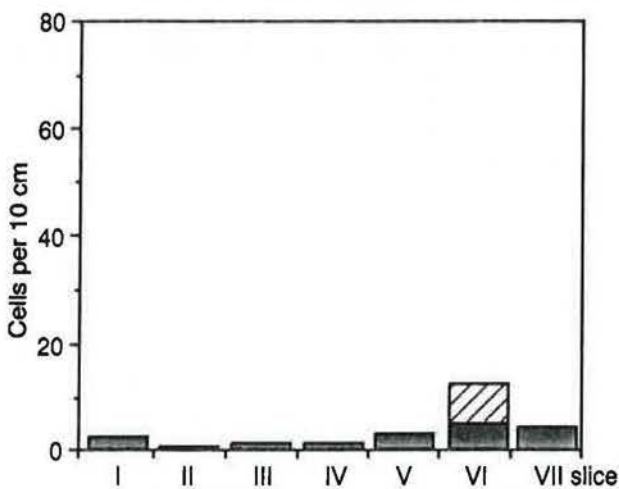


Fig. 4. - The distribution of the solitary neuroendocrine cells and neuroepithelial bodies within the mean number of neuroendocrine cells in consecutive slices of the left upper lobe in anthracosilicotic lungs. ■ : solitary neuroendocrine cells; ▨ : neuroepithelial cells.

a marked increase in the number of reactive cells in the more peripheral airways. Most peripheral NE cells (fig 3 and 4) were arranged in neuroepithelial bodies. We found no neuroepithelial bodies in the central airways (section I to III) in either anthracosilicotic (fig. 4) or control lungs (fig. 3).

Although the highest concentration of NE cells was found in the bronchioli, none was seen in the alveoli.

Discussion

The amine precursor uptake decarboxylation (APUD) system [16], based on the common property of some cells to concentrate and decarboxylate fluorogenic amines to peptide hormones, was proposed in 1966 by PEARSE [17] to encompass NE cells with similar chemical and ultrastructural characteristics. In some organs, such as the adrenal medulla and the pituitary and pancreatic islets, they become aggregated to form a recognizable endocrine structure, whereas in other organs they are scattered throughout the mucosa to form a diffuse system, as in the gastrointestinal tract and in the lung [18].

NE cells in human lungs can be found singly (fig. 5) (solitary NE cells) or in corpuscular aggregates (fig. 6), the neuroepithelial bodies [19]. These are seen as clusters of eosinophilic cells in haematoxylin-eosin stained sections, and they extend from the basement membrane of the bronchial mucosa to the lumen. Neuroepithelial bodies appear to be innervated [20] and are frequently associated with vessels. LAUWERYS and GODDEERIS [21] suggested that these bodies may act as chemoreceptors and may regulate the ventilation-perfusion balance in normal lungs. Although FEYRTER [22] described NE cells in human lungs almost half a century ago, our knowledge of the distribution and function of NE cells in human lungs has remained scanty until recently. With the development of new immunohistochemical techniques, it has become easier to study the NE cell system in human lungs. For counting these NE cells in anthracosilicotic and control lungs, we used the method of TATEISHI [13] in combination with morphometry.

We assumed that fibrosis and local hypoxia might have led to an increase in the number of NE cells in anthracosilicotic lungs. In contrast to our initial expectation we found a greater number of chromogranin-reactive cells in control than in anthracosilicotic lungs, although this difference was not statistically significant.

An advantage of the immunoperoxidase staining for chromogranin is that NE cells are stained very specifically and they can be easily recognized among the other epithelial cells. Although a limitation of the chromogranin staining is that only the granulated NE cells express this protein, comparison of our results with the study of GOSNEY *et al.* [23] reveals a similar number of NE cells (10.0 per 10 cm of bronchial epithelium) in the control population. However, both studies differ in the methods of sampling and immunostaining. In their study [23] neuron-specific enolase was used as a general NE cell marker. This confirms the usefulness of chromogranin as a NE marker in the human lung.

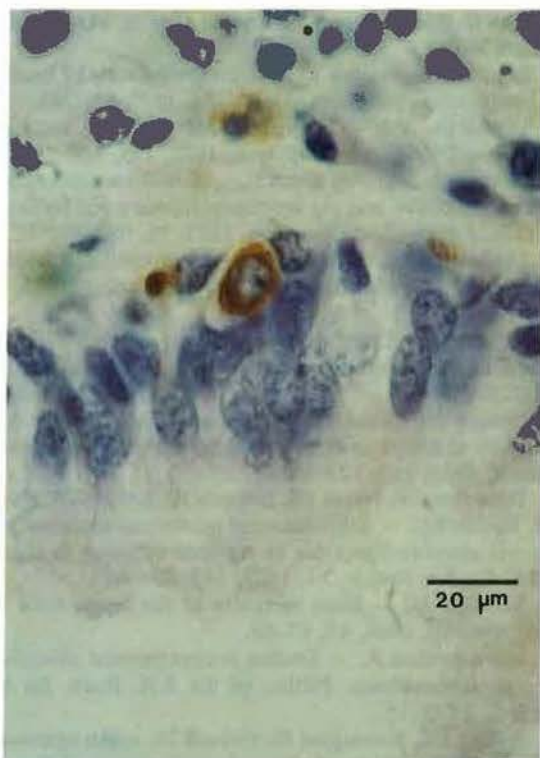


Fig. 5. - Bronchus (section II) with solitary neuroendocrine cell (arrowed) lying close to the basement membrane between other epithelial cells. (Immunoperoxidase staining for chromogranin).

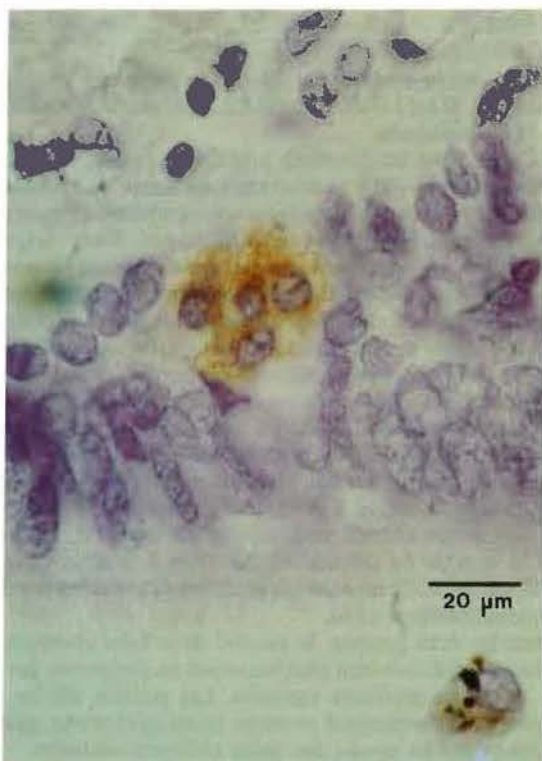


Fig. 6. - Bronchiole (section IV) with neuroepithelial body (arrowed). Notice the submucosal vessel in the neighbourhood of the neuroepithelial body. (Immunoperoxidase staining for chromogranin).

Moreover, in the lungs of hypoxic miners we found more chromogranin-reactive cells (4.4 per 10 cm of epithelium) than in miners without respiratory insufficiency (1.2 per 10 cm of epithelium), although the difference was not statistically significant. We conclude that hypoxic degranulation is not the only cause of the smaller number of chromogranin-reactive cells in anthracosilicotic lungs compared to controls.

Recent publications have shown a higher number of NE cells in human chronic bronchitis and emphysema [23], and in lungs of hamsters [24] exposed to cigarette smoke for 90 days. These findings suggest that in all of these conditions exposure to cigarette smoke is a potent stimulant of NE cell proliferation. In the present study a tendency existed for a reduction in the number of chromogranin-reactive cells in the anthracosilicotic group. This result is consistent with the hypothesis that chronic inhalation of coal-dust may destroy the NE cells in the human bronchial epithelium, as suggested by SCHUL'GA [25]. By means of experimental studies on rats he showed that anthracosis decreased the number of NE cells in the lung. As long ago as 1920, MAVROGORDATO [26] showed that silicosis had a cytotoxic effect on macrophages in the human lung. Release of multiple hydrolytic enzymes from macrophages led to the extensive fibrotic changes seen in anthracosilicotic lungs [27]. An experimental study in hamsters [24] exposed to cigarette smoke for 90 days showed an increase in the number of NE cells, indicating a clear effect of the smoke on the NE cell population.

In a study on humans with chronic bronchitis and emphysema [23] an increase in the density of NE cells was found. The most prominent increase was observed in those lobes affected by pneumonic consolidation, suggesting a possible stimulant effect of acute injury for NE cell proliferation. In fact, in the present study there was a slightly larger number of NE cells in the lungs of hypoxic miners (4.4 per 10 cm of epithelium) than in miners without respiratory insufficiency (1.2 per 10 cm of epithelium). However, this difference was not statistically significant probably due to the low number of lungs studied. A marked increase in the number of reactive NE cells was found in the more peripheral compared to the central airways in control and anthracosilicotic lungs. This result is consistent with that of TATEISHI [13], who found that NE cells were more numerous in the peripheral bronchioles with goblet cell hyperplasia. This increase in density of NE cells was predominantly caused by the appearance of neuroepithelial bodies in the more peripheral airways. Chromogranin-reactive NE cells were not found in the alveolar walls. The highest number of chromogranin-reactive neuroendocrine cells was found in the bronchioles, a very strategic place where the neuroepithelial bodies lie close to the bronchial circulation. It is not difficult to imagine that these cells secrete their neuropeptide hormones into these bronchial vessels and in this way modify the bronchial and pulmonary circulations.

Indeed, LAUWERIJNS and VAN RANST [19, 21] were able to show that hypoxia in the bronchial lumen led to degranulation of the neuroepithelial bodies. Moreover,

GOSNEY *et al.* [28] showed that the population of NE cells in human lungs is not uniform. By means of the immunoperoxidase method, they distinguished two different groups of NE cells in the human lung. The first group could be stained for gastrin-releasing peptide and the second for calcitonin by the immunoperoxidase technique. However, both groups contain pan-NE markers such as chromogranin, neuron-specific enolase and synaptophysin.

It is unknown whether each group plays a different role in the regulation of ventilation-perfusion relationships in the human lung.

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Cellules neuro-endocrines dans les poumons anthracosilicotiques. H.L.L. Gubbeldmans, B.L.P. Maesen, J. Koudstaal, F.B.J.M. Thunnissen.

RÉSUMÉ: Dans les poumons humains normaux, les cellules neuro-endocrines (NE) se trouvent sous forme de cellules NE isolées ainsi que dans des agrégats corpusculaires, qu'on appelle les corps neuro-épithéliaux. Au moyen d'une méthode morphométrique quantitative, nous avons comparé le nombre et la distribution des cellules NE réactives à la chromogranine, dans les poumons anthracosilicotiques par rapport aux poumons normaux. Après le décès, 14 paires de poumons contrôles et 12 paires de poumons anthracosilicotiques ont été sélectionnées.

L'étendue de l'anthracosilicose et de l'emphysème a été décrite au niveau du poumon droit. Le lobe supérieur gauche a été utilisé pour la détermination quantitative du nombre et de la distribution des cellules NE, visualisées grâce à une coloration immuno-histochimique à la chromogranine. A l'opposé de ce que nous avons attendu, nous n'avons pas trouvé de différence dans le nombre de cellules NE réactives à la chromogranine par 10 cm d'épithélium entre les contrôles (11.0) et les poumons anthracosilicotiques (2.4).

Dans les deux groupes, le nombre de cellules réactives à la chromogranine était bien plus important en périphérie, par rapport aux voies aériennes centrales. Les cellules NE les plus périphériques s'organisent en corps neuro-épithéliaux, que l'on n'a pas trouvé au niveau des voies aériennes centrales. *Eur Respir J.*, 1991, 4, 824–828.